Prioritization:
Chemicals Identified for Consultation with the Developmental and Reproductive Toxicant Identification Committee

August 2015

Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing five chemicals for review by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) under Proposition 65[1], using the prioritization process endorsed by the DARTIC and adopted by OEHHA in 2004. These chemicals are: nickel, pentachlorophenol, perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and tetrachloroethylene. These chemicals are not proposed for listing at this time. OEHHA is seeking public comment and the DARTIC's consultation regarding which of these chemicals should proceed to the next stage of the listing process. The next stage is the development of hazard identification materials by OEHHA followed by consideration of individual chemicals for listing by the DARTIC at future meetings.

Background

In accordance with the 2004 prioritization process, OEHHA used a focused literature review to conduct a preliminary appraisal of exposure potential and evidence of reproductive hazard on candidate chemicals in its database. The evidence of hazard used in this current round of prioritization is an epidemiologic data screen, as described below. This screen was previously applied in 2007 (http://www.oehha.ca.gov/prop65/CRNR_notices/state_listing/prioritization_notices/DARTprior090707.html), and chemicals identified as candidates were reviewed by the DARTIC at a public meeting on December 10, 2007. At public meetings of the DARTIC in 2010 and 2011, the Committee endorsed re-application of this screen in future rounds of screening.

For the current application of this epidemiologic data screen, OEHHA rescreened chemicals that had been identified in 2007 as having relevant data but which did not have sufficient human data available at that time to pass the screen. Chemicals passing the screen were then subjected to a preliminary toxicological

[1] Health and Safety Code section 25249.5 et seq.

evaluation. OEHHA is now releasing the results of the evaluation for public comment. At its November 9, 2015 meeting, the DARTIC will provide advice and consultation to OEHHA regarding possible development of hazard identification materials on the chemicals presented here. The following is a description of the process OEHHA conducted in applying this epidemiologic data screen.

Chemicals screened

OEHHA re-screened the chemicals previously identified in 2007 as having relevant but not sufficient human data to pass the screen at that time, for data suggesting that they cause reproductive and developmental effects. Ongoing potential for exposure in California was also confirmed. The evaluation of exposure potential was qualitative, based primarily on data concerning production and use of the chemical, or monitoring data. Chemicals were not screened if they are candidates for listing via an administrative listing mechanism.

Applying the epidemiology data screen

The epidemiology data screen was applied to 19 chemicals. The screen entailed the identification of chemicals with epidemiologic studies suggesting evidence of adverse developmental or reproductive effects. OEHHA conducted a literature search to identify epidemiologic studies of the chemical that report an association between exposure to the chemical and increased risk of adverse developmental or reproductive effects. More weight was given to analytical studies, and less weight to descriptive studies and case reports. Single case reports were not sufficient to satisfy the screen. For those chemicals with studies available, the abstracts were examined in detail to determine whether there was a finding of adverse developmental or reproductive effects associated with exposure to the chemical. The abstracts were further reviewed to determine whether the effect might be attributed with some confidence to exposure to the chemical of concern. Two or more analytical studies of adequate quality were required for the chemical to pass the screen.

Preliminary toxicological evaluation

After applying the epidemiologic data screen, OEHHA then conducted a preliminary evaluation of the animal toxicology data for the chemicals identified. This involved a further search of the literature to identify animal reproductive or developmental toxicity studies, studies on the mechanism of action, metabolism, and pharmacokinetics. This additional information was used in conducting a preliminary evaluation of the overall evidence of reproductive or developmental toxicity for each of the chemicals identified by the epidemiology data screen. Chemicals for which the preliminary evaluation indicated that developmental and

reproductive toxicity might be a concern are being proposed here for DARTIC consideration.

Chemicals proposed for DARTIC consideration

The above process yielded five chemicals: nickel, pentachlorophenol, perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and tetrachloroethylene.

The Appendix provides an explanation of why each of the five chemicals was chosen. Briefly, for each chemical chosen there were available at least two analytical epidemiologic studies of adequate quality reporting an association between exposure to the chemical and increased risk of adverse developmental or reproductive outcomes (the basic criterion for selection), plus additional relevant studies in humans and animals. The number of analytical epidemiologic studies of adequate quality reporting such an association for each chemical was:

nickel:	three
pentachlorophenol:	four
PFOA:	twenty
PFOS:	fifteen
tetrachloroethylene:	five

Abstracts of the epidemiological studies, animal toxicity studies, and other data relevant to the reproductive or developmental toxicity that were identified during this process for each of these five chemicals have been included verbatim in the Appendix, when available. These abstracts were obtained from sources such as on-line journals and PubMed.

Next Steps

With the publication of this document on August 28, 2015, OEHHA opened the public comment period on the chemicals proposed for DARTIC consideration. The comment period closes on October 12, 2015.

The DARTIC will discuss the five chemicals proposed for its consideration at its November 9 meeting, and provide advice and consultation regarding possible development of hazard identification materials. Written public comments received by OEHHA by October 12, 2015, will be provided to the DARTIC for review.

At the DARTIC meeting, the public will be given the opportunity to comment on the chemicals being proposed for possible hazard identification materials preparation. The DARTIC could vote on recommendations or provide less formal

advice to OEHHA concerning which chemicals should be brought back for future consideration for listing. In addition, the DARTIC may also suggest other chemicals for which hazard identification materials should be prepared.

Hazard identification materials summarizing the available scientific evidence on the developmental and reproductive toxicity potential of the selected chemicals will be prepared, based on an extensive search of the scientific literature. OEHHA will provide these materials to the DARTIC and release them for public comment prior to the public meeting at which the DARTIC will consider the chemical's listing.

Further details on prioritization, the development of hazard identification materials, and committee consideration of the listing of chemicals under Proposition 65 are given in the OEHHA document cited below.

Reference

Office of Environmental Health Hazard Assessment (OEHHA, 2004). *Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts."* California Environmental Protection Agency, OEHHA, Sacramento, CA, December. Available online at: www.oehha.ca.gov/prop65/CRNR_notices/state_listing/pdf/finalPriordoc.pdf

APPENDIX: NICKEL

Nickel (CAS# 7440-02-0) is a very abundant natural element, used mostly to make stainless steel. Nickel compounds are used for nickel plating, to make some batteries, and as substances known as catalysts for chemical reactions.

This document presents a compilation of abstracts of articles on the developmental and reproductive toxicity of nickel identified during our epidemiological screen and subsequent toxicological evaluation. OEHHA originally screened nickel in 2007 but there was not sufficient human data available for nickel to pass the screen at that time. We applied an epidemiologic data screen on nickel in 2015. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. Nickel passed the epidemiologic screen. We also conducted a preliminary toxicological evaluation searching for relevant studies, including animal studies.

OEHHA used the information in this document to select nickel for presentation to the Developmental and Reproductive Toxicant Identification Committee as a possible candidate for Committee consideration. The abstracts compiled below are from epidemiologic and animal toxicity studies reporting on developmental and reproductive sequelae related to exposure to nickel, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species).

Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Seven epidemiologic studies of nickel reporting statistically significant increased risk of adverse developmental or reproductive outcomes were identified, three of which were analytical studies of adequate quality. One additional epidemiologic study reported increased risk of adverse developmental or reproductive outcome; however, the findings were not statistically significant. Eleven epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified, as well as one study which had unclear findings. Twenty-one related articles and four studies without an abstract were also identified.

In addition, the following animal toxicity studies were identified.

- Thirty-five animal studies of nickel reporting reproductive or developmental toxicity were identified, as well as five studies reporting no reproductive or developmental toxicity. Sixty-one related articles and eighteen studies without abstracts were identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

i. Studies which were statistically significant

***Airborne PM2.5 chemical components and low birth weight in the northeastern and mid-Atlantic regions of the United States**.
Ebisu K. and Bell M. L.
Environ Health Perspect. 2012;120(12):1746-52.

BACKGROUND: Previous studies on air pollutants and birth outcomes have reported inconsistent results. Chemical components of particulate matter ≤ 2.5 μm (PM2.5) composition are spatially heterogeneous, which might contribute to discrepancies across PM2.5 studies.
OBJECTIVES: We explored whether birth weight at term is affected by PM2.5, PM10 (PM ≤10 μm), and gaseous pollutants.
METHODS: We calculated exposures during gestation and each trimester for PM2.5 chemical components, PM10, PM2.5, carbon monoxide, nitrogen dioxide, ozone, and sulfur dioxide for births in 2000-2007 for states in the northeastern and mid-Atlantic United States. Associations between exposures and risk of low birth weight (LBW) were adjusted by family and individual characteristics and region. Interaction terms were used to investigate whether risk differs by race or sex.
RESULTS: Several PM2.5 chemical components were associated with LBW. Risk increased 4.9% (95% CI: 3.4, 6.5%), 4.7% (3.2, 6.2%), 5.7% (2.7, 8.8%), and 5.0% (3.1, 7.0%) per interquartile range increase of PM2.5 aluminum, elemental carbon, nickel, and titanium, respectively. Other PM2.5 chemical components and gaseous pollutants showed associations, but were not statistically significant in multipollutant models. The trimester associated with the highest relative risk differed among pollutants. Effect estimates for PM2.5 elemental carbon and nickel were higher for infants of white mothers than for those of African-American mothers, and for males than females.
CONCLUSIONS: Most exposure levels in our study area were in compliance with U.S. Environmental Protection Agency air pollution standards; however, we identified

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

associations between PM2.5 components and LBW. Findings suggest that some PM2.5 components may be more harmful than others, and that some groups may be particularly susceptible.

Effect of exposure to trace elements in the soil on the prevalence of neural tube defects in a high-risk area of China.
Huang J., Wu J., Li T., Song X., Zhang B., Zhang P. and Zheng X.
Biomed Environ Sci. 2011;24(2):94-101.

OBJECTIVE: Our objective is to build a model that explains the association between the exposure to trace elements in the soil and the risk of neural tube defects.
METHODS: We built a function with different parameters to describe the effects of trace elements on neural tube defects. The association between neural tube defects and trace element levels was transformed into an optimization problem using the maximum likelihood method.
RESULTS: Tin, lead, nickel, iron, copper, and aluminum had typical layered effects (dosage effects) on the prevalence of neural tube defects. Arsenic, selenium, zinc, strontium, and vanadium had no effect, and molybdenum had one threshold value that affected the prevalence of birth defects.
CONCLUSION: As an exploratory research work, our model can be used to determine the direction of the effect of the trace element content of cultivated soil on the risk of neural tube defects, which shows the clues by the dosage effect of their toxicological characteristics. Based on our findings, future biogeochemical research should focus on the direct effects of trace elements on human health.

***Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources**.
Bell M. L., Belanger K., Ebisu K., Gent J. F., Lee H. J., Koutrakis P. and Leaderer B. P.
Epidemiology. 2010;21(6):884-91.

BACKGROUND: Exposure to fine particles (PM2.5) during pregnancy has been linked to lower birth weight; however, the chemical composition of PM2.5 varies widely. The health effects of PM2.5 constituents are unknown.
METHODS: We investigated whether PM2.5 mass, constituents, and sources are associated with birth weight for term births. PM2.5 filters collected in 3 Connecticut

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counties and 1 Massachusetts county from August 2000 through February 2004 were analyzed for more than 50 elements. Source apportionment was used to estimate daily contributions of PM2.5 sources, including traffic, road dust/crustal, oil combustion, salt, and regional (sulfur) sources. Gestational and trimester exposure to PM2.5 mass, constituents, and source contributions were examined in relation to birth weight and risk of small-at-term birth (term birth < 2500 g) for 76,788 infants.
RESULTS: Road dust and related constituents such as silicon and aluminum were associated with lower birth weight, as were the motor-vehicle-related species such as elemental carbon and zinc, and the oil-combustion-associated elements vanadium and nickel. An interquartile range increase in exposure was associated with low birthweight for zinc (12% increase in risk), elemental carbon (13%), silicon (10%), aluminum (11%), vanadium (8%), and nickel (11%). Analysis by trimester showed effects of third-trimester exposure to elemental carbon, nickel, vanadium, and oil-combustion PM2.5.
CONCLUSIONS: Exposures of pregnant women to higher levels of certain PM2.5 chemical constituents originating from specific sources are associated with lower birth weight.

***Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China**.
Guo Y., Huo X., Li Y., Wu K., Liu J., Huang J., Zheng G., Xiao Q., Yang H., Wang Y., Chen A. and Xu X.
Sci Total Environ. 2010;408(16):3113-7.

Toxic heavy metals are released to the environment constantly from unregulated electronic waste (e-waste) recycling in Guiyu, China, and thus may contribute to the elevation of lead and other heavy metals levels in placenta. We aimed to investigate concentrations of heavy metals, including lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) in placenta from Guiyu and compared them with those from a control area where no e-waste processing occurs. Two hundred and twenty human placentas were collected from Guiyu (n=101) and the control area (n=119). The placenta concentrations of Pb, Cd, Cr, and Ni (PCPb, PCCd, PCCr, and PCNi) were determined by graphite furnace atomic absorption spectrometry (GFAAS). Risk factors of high exposure and correlation with adverse pregnancy outcomes were analyzed using Spearman correlation analyses. PCPb from Guiyu ranged from 6.51 to 3465.16ng/g with a median of 301.43ng/g, whereas PCPb from the control area ranged from 4.53 to 3176.12ng/g

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with a median of 165.82ng/g (P=0.010). We also observed that in Guiyu, 41.6% of women (42/101) had PCPb > 500ng/gwt (wet weight), compared with 24.4% of women (29/119) in the control area (P=0.006). No significant differences of PCCd and PCCr were found between the two groups. In contrast, PCNi was higher in samples from the control area (median 14.30, range 1.76-593.70ng/g) than in Guiyu samples (median 7.64, range 1.19-1108.99ng/g) (P=0.000), and a negative correlation between PCNi and gestational age was found in this study (P=0.017). Spearman correlation analyses showed that there was correlation between PCPb and residence in e-waste recycling area. Environmental pollution, resulted from unregulated e-waste recycling activities, may contribute to elevated PCPb in neonates born in Guiyu and threaten their health.

The prevalence of selected pregnancy outcome risk factors in the life-style and medical history of the delivering population in north-western Russia.
Vaktskjold A., Paulsen E. E., Talykova L., Nieboer E. and Odland J. O.
Int J Circumpolar Health. 2004;63(1):39-60.

OBJECTIVES: A population-based birth registry has been set up for the Arctic town of Moncegorsk in north-western Russia. This investigation describes the health status of the delivering population, including pregnancy history and the prevalence of obesity, infections, smoking and alcohol abuse during the pregnancy period. An overview of the occupations of the delivering population is also presented. METHODS: The birth registry contains detailed and verified information about the newborn, delivery, pregnancy and the mother for 21,214 births by women from Moncegorsk in the period 1973-97. RESULTS: Of the delivering women, 15.7% had experienced one or more spontaneous abortions, and 47.4% had at least one induced abortion. More than 9% had suffered pelvic inflammatory disease (PID) in their past. The local nickel company employed 9016 (42.5%) of the delivering women; of these 17% worked in production areas with exposures to compounds of nickel, among other hazards, and 38% are judged to have had possible, or probable, exposure of this type. CONCLUSION: Compared with the delivering population in Norway, that in Moncegorsk was younger and had a lower prevalence of obesity, diabetes and heavy smoking. The most worrisome findings were the high prevalence of a history of abortion and PID. A relatively high proportion of the women worked in physically demanding, or/and nickel-exposed occupations.

Semen quality of Indian welders occupationally exposed to nickel and chromium.
Danadevi K., Rozati R., Reddy P. P. and Grover P.
Reprod Toxicol. (Elmsford, NY). 2003;17(4):451-6.

The semen quality of 57 workers from a welding plant in South India and 57 controls was monitored. Blood nickel and chromium concentrations were determined by ICP-MS. Analysis of semen samples was performed in accordance with World Health Organization criteria. The blood level of nickel and chromium for the 28 exposed workers was 123.3 +/- 35.2 and 131.0 +/- 52.6 microg/l, resepctively, which was significantly higher than the 16.7 +/- 5.8 and 17.4 +/- 8.9 microg/l for the control group (n=27). Sperm concentrations of exposed workers were 14.5 +/- 24.0 millions/ml and those of the control group were 62.8 +/- 43.7 millions/ml. Rapid linear sperm motility was decreased in exposed workers compared to controls. There was a significant positive correlation between the percentage of tail defects and blood nickel concentration in exposed workers. The sperm concentration showed a negative correlation with blood chromium content in workers. More abnormal characteristics were found in the semen of exposed workers. Semen abnormalities correlated with the number of years of exposure to welding fumes containing nickel and chromium.

Congenital defects, abortion and other health effects in nickel refinery workers.
Chashschin V. P., Artunina G. P. and Norseth T.
Sci Total Environ. 1994;148(2-3):287-91.

Health impairment was investigated by a thorough clinical investigation in a cross-sectional study of 821 male and 758 female workers in a nickel hydrometallurgy refining plant. The average nickel exposure levels were found to be around 0.2 mg/m3 in the electrolysis department and 0.13 mg/m3 in the electrolyte purification department. Corresponding average urinary values for nickel were 16 micrograms/l and 10 micrograms/l, respectively. The most common types of health impairment found were respiratory, skin and cardiovascular diseases. Health impairments, except for respiratory diseases, were found more often in females than in males. The design of the study does not allow comparison with a non-exposed population. Even if there are serious limitations in the statistical and sampling details of the pregnancies and new-born babies, the results suggest adverse health effects at usually accepted exposure levels to nickel. Normal pregnancies were reported in 29% of 356 pregnant nickel workers compared with 39% in 342 local construction workers. Spontaneous and threatening abortions were reported in 16% and 17% of all pregnancies in nickel-exposed workers, compared with 9% and 8%, respectively in the construction workers.

Structural malformations were found in about 17% of alive-born infants with nickel-exposed mothers, compared with about 6% in the reference group. Significant increased risks of 2.9, 6.1 and 1.9 for total defects, cardiovascular defects and defects of the musculoskeletal system, respectively, were demonstrated.

ii. Studies which were not statistically significant

Spontaneous abortions among nickel-exposed female refinery workers.
Vaktskjold A., Talykova L. V., Chashchin V. P., Odland J. O. and Nieboer E.
Int J Environ Health Res. 2008;18(2):99-115.

A case-control study to investigate whether women employed in nickel-exposed work areas in early pregnancy are at elevated risk of spontaneous abortion (SA). Data about pregnancy outcome and maternal factors were obtained about each delivery and SA from women in selected work places. Each pregnancy record was assigned a categorical nickel (Ni) exposure rating according to the women's occupations at pregnancy onset. The guidelines were the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers or/and measured urinary-Ni concentrations. The unadjusted odds ratio for the association between the maternal exposure to Ni and an SA for Ni-exposed women was 1.38 (95% confidence interval: 1.04-1.84), and the adjusted was 1.14 (0.95-1.37). In conclusion, there was no statistical association between maternal occupational exposure to water-soluble Ni in early pregnancy and the risk of self-reported SA. The findings do not exclude the possibility of a weak excess risk, or a risk in the first weeks of pregnancy.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Does the metal content in soil around a pregnant woman's home increase the risk of low birth weight for her infant?
McDermott S., Bao W., Aelion C. M., Cai B. and Lawson A. B.
Environ Geochem Health. 2014;36(6):1191-7.

Low birth weight (LBW) is associated with a number of maternal environmental exposures during pregnancy. This study explored the association between soil metal

concentrations around the home where the mother lived during pregnancy and the outcome of LBW. We used a retrospective cohort of 9,920 mother-child pairs who were insured by Medicaid during pregnancy and lived in ten residential areas, where we conducted soil sampling. We used a grid that overlaid the residential areas and collected soil samples at the grid intersections. The soil was analyzed for the concentration of eight metals [arsenic (As), barium (Ba), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), and mercury (Hg)], and we then used Bayesian Kriging to estimate the concentration at the actual maternal addresses, since we had the GIS coordinates of the homes. We used generalized additive modeling, because the metal concentrations had nonlinear associations with LBW, to develop the best fitting multivariable model for estimating the risk of LBW. The final model showed significant associations for female infants, maternal smoking during pregnancy, non-white mothers, Cu, and As with LBW. The As variable was nonlinear in relation to LBW, and the association between higher concentrations of As with LBW was strong ($p = 0.002$). We identified a statistically significant association between soil concentrations of arsenic around the home of pregnant women and an increased risk of LBW for her infant.

Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study.
Zheng G., Zhong H., Guo Z., Wu Z., Zhang H., Wang C., Zhou Y. and Zuo Z.
Biol Trace Elem Res. 2014;160(3):437-44.

To better understand the relationship between prenatal exposure to heavy metals and trace elements and the risk of adverse pregnancy outcomes, we investigated the status of heavy metals and trace elements level in a Chinese population by collecting umbilical cord blood. Umbilical cord blood heavy metals and trace elements concentrations were determined by inductively coupled plasma-mass spectrometry. No differences with statistical significance in the median arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), strontium (Sr), thallium (Tl), vanadium (V), and zinc (Zn) concentrations were observed between the adverse pregnancy outcome group and the reference group. Titanium (Ti) and antimony (Sb) were found at higher levels with statistical significance in the cord blood samples with adverse pregnancy group when compared to the ones in the reference group. The association between Ti levels and the risk of adverse pregnancy outcomes remained significant after adjusting for potential confounding factors, including newborn weight. These results indicated that environmental exposure to Ti may increase the risk of adverse pregnancy outcomes in Chinese women without occupational exposure.

Associations between blood metals and fecundity among women residing in New York State.
Bloom M. S., Louis G. M., Sundaram R., Kostyniak P. J. and Jain J.
Reprod Toxicol. 2011;31(2):158-63.

Trace exposures to metals may affect female reproductive health. To assess the relation between trace concentrations of blood metals and female fecundity, 99 non-pregnant women discontinuing contraception for the purpose of becoming pregnant were prospectively followed. Participants completed a baseline interview and daily diaries until pregnant, or up to 12 menstrual cycles at risk for pregnancy; home pregnancy test kits were used. For 80 women, whole blood specimens were analyzed for arsenic, cadmium, lead, magnesium, nickel, selenium and zinc using inductively coupled plasma mass spectrometry (ICP-MS). Time to pregnancy was estimated using Cox proportional hazards models for discrete time. Metal concentrations were generally within population reference intervals. Adjusted models suggest a 51.5% increase in the probability for pregnancy per 3.60 µg/L increase in Mg (P=0.062), and a 27.7% decrease per 0.54 µg/L increase in Zn (P=0.114). Findings indicate that Mg and Zn may impact female fecundity, but in varying directions.

[Influence of nonindustrial risk factors on congenital abnormalities formation].
Talykova L. V.
Med Tr Prom Ekol. 2009;5:35-8.

Congenital abnormalities could result from exposure to occupational hazards. Epidemiologic study of nickel compounds influence on reproductive health in females engaged into nickel purification in Murmansk region enterprises did not reveal increased risk of the anomalies. The study was aimed to define influence of various risk factors connected not to work conditions, but to mother's health, bad habits, age, on congenital abnormalities in newborns.

Maternal nickel exposure and congenital musculoskeletal defects.
Vaktskjold A., Arild V., Talykova L. V., Ljudmila V. T., Chashchin V. P., Valerij P. C., Odland J. O., Jon O. O., Nieboer E. and Evert N.
Am J Ind Med. 2008;51(11):825-33.

OBJECTIVE: To investigate whether women occupationally exposed to nickel in early pregnancy are at elevated risk of delivering a newborn with a malformation or deformation of the musculoskeletal system (ICD-10: Q65-Q79).

METHODS: Data about the newborn, maternal occupation and workplace were obtained using the Kola Birth Register (KBR). Each record in the KBR was assigned a categorical nickel (Ni) exposure rating according to the occupation the delivering woman had at the time of becoming pregnant. This was achieved by using as a guideline the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers or/and measured urinary-Ni concentrations. The reference population was delivering women from the source population with background exposure level. In total, the study population consisted of 22,965 births.
RESULTS: Three hundred and four infants (13.3/1,000 births; 95% confidence interval (CI): 11.9-14.7) were diagnosed with isolated musculoskeletal defect(s) at birth. The adjusted odds ratio for the association between the maternal exposure to Ni and this outcome was 0.96 (95% CI: 0.76-1.21) per unit increase in exposure category.
CONCLUSION: The incidence of defects in the musculoskeletal system at birth was high, especially for feet deformities, but we found no effect of maternal exposure to water-soluble Ni on the risk of delivering a newborn with a defect. However, the incidence among women working in the copper refinery was higher than in the other employment groups.

Small-for-gestational-age newborns of female refinery workers exposed to nickel.
Vaktskjold A., Talykova L. V., Chashchin V. P., Odland J. O. and Nieboer E.
Int Occ Med Environ Health. 2007;20(4):327-38.

OBJECTIVES: It has not yet been established whether exposure to nickel (Ni) compounds may cause reproductive toxicity. The objective of this study was to investigate whether women employed under conditions of nickel exposure in early pregnancy were at elevated risk of delivering a newborn small-for-gestational-age (SGA).
MATERIALS AND METHODS: A register-based study of a well defined population. Data on pregnancy outcome and maternal occupation were obtained from the Kola Birth Registry. Each birth record was assigned a Ni exposure rating category according to maternal occupation at the time of becoming pregnant. Nickel exposure assessment was based on determining the water-soluble Ni subfraction of respirable aerosol fraction obtained by personal monitoring, and/or on measurements of urine Ni concentration. The reference population were the delivering women with background exposure level. The study population consisted of 22 836 births (> 27 weeks of gestation) and the SGA infants were defined as below the 10th percentile birth weight for gestational age in the

source population. Multiple logistic regression was used to analyze the association of the outcome with the assigned exposure rating category.
RESULTS: The adjusted odds ratio for Ni-exposed women for giving birth to an SGA newborn was 0.84 (95% CI: 0.75-0.93).
CONCLUSIONS: We found no adverse effect of maternal occupational exposure to water-soluble Ni in the first part of pregnancy on the risk of delivering an SGA newborn without trisomy. The finding does not exclude a possibility that exposure throughout pregnancy might produce such an effect.

Genital malformations in newborns of female nickel-refinery workers.
Vaktskjold A., Talykova L. V., Chashchin V. P., Nieboer E., Thomassen Y. and Odland J. O.
Scan J Work, Environ Health. 2006;32(1):41-50.

OBJECTIVES: This study investigated whether pregnant women employed in nickel-exposed work areas are at elevated risk of delivering a newborn with a genital malformation.
METHODS: In this register-based cohort study, data about pregnancy outcome and occupation were obtained using the Kola Birth Registry. Each record in the Registry was assigned a categorical nickel exposure rating according to the occupation the delivering woman had at the time of becoming pregnant, using, as guidelines, the water-soluble nickel subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel-refinery workers or the measured urinary nickel concentrations. The reference population comprised delivering women from Moncegorsk with a background exposure level. The association of the outcome with the assigned exposure ratings was analyzed in a logistic regression model, adjusted for parity, maternal malformation, exposure to solvents, and infection in early pregnancy.
RESULTS: The odds ratio for nickel-exposed women delivering a newborn with a genital malformation was 0.81 [95% confidence interval (95% CI) 0.52-1.26], and that for an undescended testicle was 0.76 (95% CI 0.40-1.47).
CONCLUSIONS: In this study no negative effect of maternal exposure to water-soluble nickel was found on the risk of delivering a newborn with malformations of the genital organs. The results should be interpreted with caution since there were few cases in the higher exposure groups. The findings do not exclude the possibility of an effect on the risk of other congenital malformations and adverse outcomes (including reduced fertility).

Elements in placenta and pregnancy outcome in arctic and subarctic areas.
Odland J. O., Nieboer E., Romanova N. and Thomassen Y.
Int J Circumpolar Health. 2004;63(2):169-87.

OBJECTIVES: This paper describes a comprehensive assessment of the association of concentrations of essential and toxic elements in maternal and neonatal body fluids and the placenta as predictors of birth weight and newborn body mass index (BMIC) for deliveries in northern Norway and Russia.
STUDY DESIGN: A prospective cross-sectional study of delivering women and their outcomes from different locations in Russian and Norwegian arctic and sub-arctic areas.
METHODS: Life-style information, blood, urine and placenta specimens were collected for 50 consecutive mother-infant pairs from hospital delivery departments in a total of six communities located in Finnmark, Norway, or the western arctic/subarctic regions of Russia. Questionnaire information was collected by individual interviews performed by trained health personnel. Pregnancy outcomes were verified by consulting medical records. Cadmium, copper, iron (as ferritin), nickel, lead, selenium and zinc were measured in maternal blood, serum or maternal urine: and in cord blood, or neonatal urine and placental tissue. Univariate and multivariate linear regression analysis and ANOVA were employed to explore associations between these clinical chemistry outcomes and birth weight and BMIC.
RESULTS: A number of significant relationships were evident between: placental and maternal blood cadmium ($p < 0.005$); cord and maternal blood lead ($p < 0.001$); placental and maternal blood lead ($p < 0.001$); placental and cord-blood lead ($p < 0.001$); placental and maternal serum, or blood, selenium ($p < 0.001$); and placental and maternal serum copper ($p < 0.001$). Maternal body mass index (BMI), maternal age, placental lead, or maternal blood lead, and smoking were retained as predictors of birth weight and BMIC in the multivariate modelling. Birth weights in both countries were normally distributed.
CONCLUSIONS: Maternal age and BMI as positive predictors of birth weight, and cigarette smoking and lead exposure as negative determinants, are discussed in terms of established evidence and recognized confounders, including maternal genetic factors, socio-economic status, socio-political change, life-style issues, prenatal care and nutrition. It is recommended that future work in societies undergoing socio-economic transition might best focus on preventive measures to improve neonatal health and development.

Urinary nickel concentrations and selected pregnancy outcomes in delivering women and their newborns among arctic populations of Norway and Russia.
Odland J. O., Nieboer E., Romanova N., Thomassen Y., Norseth T. and Lund E.
J Environ Monit. 1999;1(2):153-61.

The two objectives of this study were to compare urinary nickel excretion in pregnant women and their newborns living in the Murmansk and Arkhangelsk Counties of Russia with that in comparable Norwegian populations living in Finnmark and the city of Bergen and to assess the influence on pregnancy outcome of different risk variables, specifically urinary nickel concentrations and questionnaire-based anamnestic information. Life-style information and urine samples were collected from 50 consecutive mother-infant pairs from hospital delivery departments in three Russian and three Norwegian communities. Pregnancy outcomes were verified from medical records. Urinary nickel excretion was significantly higher in the Russian communities, independent of the presence of a nickel refinery as a local environmental source. The birth weight and mean body mass index of the newborn children (BMIC) were significantly lower ($p < 0.001$) in the Russian groups, with or without adjustment for gestational age. A multivariate linear regression analysis indicated that maternal urinary nickel concentration had no impact on birth weight. The maternal body mass index (BMI) and maternal height were positive explanatory variables; maternal urinary creatinine is suggested as a weak negative factor. Smoking was shown to be a strong negative predictor only in the Norwegian group among whom there was a significantly higher smoking frequency ($p = 0.005$). The significant contribution of a country factor in the predictive model is interpreted to indicate that a number of important risk factors for low birth weight were not identified.

Heavy metals (Pb, Cd, Ni) concentration in the hair of mothers of preterm and small for gestational age (SGA) infants.
Peitrzyk J. J., Nowak A., Mitkowska Z., Petko M., Zachwiejowa Z., Chelopicka J., Kroasniak M., Glianska A., Strzelecki T., Dobosz P. and et al.
Pediatr Res. 1994;36(1 Pt 2).

To test null hypothesis (Ho): prenatal exposure to heavy metals does not increase the risk of prematurity and the delivery of small for gestational (SGA) infants, a case-control study was carried out in Southern Poland. Material: From July 1992 through June 1993 all cased of SGA (less than 10 perc.) (N = 74) and preterm (less than 37 wks) newborns

(N = 104) were ascertained prospectively in 4 regions (Krak„ow, Zakopane, Limanowa, Rabka). For each case at least one control infant (greater than 10 perc. and greater than 37 wks) matched by sex and birth-date was selected (N = 211). Case and control mothers' pubic and head hair were collected. Methods: Pb, Ni and Cd were determined in hair samples by atomic absorption spectrometry (Perkin Elmer). Results: Case control analysis (ANOVA) of Pb, Ni and Cd revealed that Cd content was significantly increased in the head hair of mothers of SGA infants (f = 7.49 p = 0.007). Also mothers of preterm newborns showed significantly higher Pb concentration in pubic hair in comparison to the controls (F = 4.67 p = 0.03). No significant case/control difference was observed for Ni. Conclusion: Increased Cd and Pb content in maternal hair reflects higher exposure to these metals, which may be related to higher risk of preterm and SGA infants delivery.

Stainless steel welding and semen quality.
Jelnes J. E. and Knudsen L. E.
Reprod Toxicol. 1988;2(3-4):213-5.

Questionnaire studies of patients from fertility clinics suggest that welders may have an increased risk of reduced semen quality. In this study, welders and nonwelders from the same plants were asked to provide blood, urine, and semen samples. Urine was analyzed for chromium and nickel, and for mutagenic activity and metal concentration; blood for metal concentrations, immunoglobulin G, total protein, and measures of genotoxicity in lymphocytes; and semen was evaluated by standard semen analysis. Results of the semen evaluation, presented here, showed no difference in semen quality between welders and nonwelders. Because the metal dust exposure of nonwelders in the plant may be higher than that in the general population, welders were also compared to referents not working in the metal industry. Again, no decrease in semen quality associated with welding was demonstrated.

C. Studies with unclear findings

Autism spectrum disorders in relation to distribution of hazardous air pollutants in the San Francisco bay area.
Windham G. C., Zhang L., Gunier R., Croen L. A. and Grether J. K.
Environ Health Perspect. 2006;114(9):1438-44.

OBJECTIVE: To explore possible associations between autism spectrum disorders (ASD) and environmental exposures, we linked the California autism surveillance system to estimated hazardous air pollutant (HAP) concentrations compiled by the U.S. Environmental Protection Agency.
METHODS: Subjects included 284 children with ASD and 657 controls, born in 1994 in the San Francisco Bay area. We assigned exposure level by census tract of birth residence for 19 chemicals we identified as potential neurotoxicants, developmental toxicants, and/or endocrine disruptors from the 1996 HAPs database. Because concentrations of many of these were highly correlated, we combined the chemicals into mechanistic and structural groups, calculating summary index scores. We calculated ASD risk in the upper quartiles of these group scores or individual chemical concentrations compared with below the median, adjusting for demographic factors.
RESULTS: The adjusted odds ratios (AORs) were elevated by 50% in the top quartile of chlorinated solvents and heavy metals [95% confidence intervals (CIs) , 1.1-2.1], but not for aromatic solvents. Adjusting for these three groups simultaneously led to decreased risks for the solvents and increased risk for metals (AORs for metals: fourth quartile = 1.7 ; 95% CI, 1.0-3.0 ; third quartile = 1.95 ; 95% CI, 1.2-3.1) . The individual compounds that contributed most to these associations included mercury, cadmium, nickel, trichloroethylene, and vinyl chloride.
CONCLUSIONS: Our results suggest a potential association between autism and estimated metal concentrations, and possibly solvents, in ambient air around the birth residence, requiring confirmation and more refined exposure assessment in future studies.

D. Related articles

Associations of neonatal lead, cadmium, chromium and nickel co-exposure with DNA oxidative damage in an electronic waste recycling town.
Ni W., Huang Y., Wang X., Zhang J. and Wu K.
Sci Total Environ. 2014;472:354-62.

OBJECTIVE: This study aimed to evaluate the effects of toxic heavy metal co-exposure on DNA oxidative damage in neonates from a primitive e-waste recycling region, Guiyu town, China.
METHODS: Our participants included 201 pregnant women: 126 from Guiyu town and 75 from Jinping district of Shantou city, where no e-waste recycling and dismantling activities existed. Structured interview questionnaires were administered to the pregnant women and umbilical cord blood (UCB) samples were collected after delivery. The UCB concentrations of lead, cadmium, chromium, and nickel were analyzed by graphite furnace atomic absorption spectrometry (GFAAS). Levels of UCB plasma 8-hydroxydeoxyguanosine (8-OHdG, a DNA oxidative damage biomarker) were determined by enzyme-linked immunosorbent assay.
RESULTS: Our results suggested that UCB lead and cadmium concentrations in neonates of Guiyu were significantly higher than those of Jinping (lead: median 110.45 ng/mL vs. 57.31 ng/mL; cadmium: median 2.50 ng/mL vs. 0.33 ng/mL, both $P < 0.001$). Parents' residence in Guiyu, and parents' work related to e-waste recycling were the risk factors associated with neonate's UCB lead and cadmium levels. No significant difference of UCB plasma 8-OHdG levels was found between Guiyu and the control area. After adjusting for potential confounders, cord plasma 8-OHdG concentrations (ng/mL) were positively associated with blood cadmium (β=0.126 ng/mL, 95% CI: 0.055 to 0.198 ng/mL), chromium (β=0.086 ng/mL, 95% CI: 0.014 to 0.158 ng/mL) and nickel (β=0.215 ng/mL, 95% CI: 0.113 to 0.317 ng/mL) concentrations.
CONCLUSIONS: The primitive e-waste recycling and dismantling activities may contribute to the elevated umbilical cord blood toxic heavy metal levels in neonates born in Guiyu. Exposures to cadmium, chromium and nickel were associated with increased oxidative DNA damage in neonates.

Assessment of exposure to trace metals in a cohort of pregnant women from an urban center by urine analysis in the first and third trimesters of pregnancy.
Fort M., Cosin-Tomas M., Grimalt J. O., Querol X., Casas M. and Sunyer J.
Environ Sci Pollut Res Int. 2014;21(15):9234-41.

Prenatal exposure to trace metals, whether they are essential, non-essential, or toxic, must be assessed for their potential health effects in the offspring. Herein is reported an approach to this end which involved collection of urine samples during the first and third trimesters of pregnancy from 489 mothers from Sabadell (Catalonia, Spain), a highly industrialized town. These samples were analyzed for cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), arsenic (As), molybdenum (Mo), cadmium (Cd), antimonium (Sb), cesium (Cs), thallium (Tl), and lead (Pb). An acid digestion method was developed and validated for inductively coupled plasma quadruple mass spectrometry (Q-ICP-MS) analysis of these 12 metals. The median concentrations of metals ranged from 0.13 to 290 mug/g creatinine, the highest levels were found for Zn and the lowest for Th. The mean concentrations of most metals except As, Ni, Th, and Pb showed statistically significant differences between both trimesters. The concentrations of Mo, Se, Cd, Cs, and Sb were higher in the first than in the third trimester, whereas the opposite was found for Co, Cu, and Zn. The concentrations of all metals in both sampling periods showed statistically significant correlations ($p<0.01$ for Mo and Cu, $p<0.001$ for the others). The significant correlations of metal urine concentrations in the first and third trimesters of pregnancy suggest that the observed differences between both periods are related to physiological changes. Accordingly, the measured urine concentrations during either the first or third trimesters can be used as estimates of exposure during pregnancy and can serve as markers for prenatal intake of these metals in the studied cohort.

Metal ion levels in maternal and placental blood after metal-on-metal total hip arthroplasty.
Novak C. C., Hsu A. R., Della Valle C. J., Skipor A. K., Campbell P., Amstutz H. C., Jiranek W. A., Onyike A., Pombar X. F. and Jacobs J. J.
Am J Orthop (Belle Mead, NJ). 2014;43(12):E304-8.

There is concern regarding elevated metal ion levels in the blood during pregnancy and the potential fetal effects in women with metal-on-metal (MOM) implants. We obtained maternal and umbilical cord blood samples from 3 patients with a MOM hip arthroplasty and 7 control subjects without any metallic implants. Serum metal ion levels including chromium, cobalt, titanium, and nickel were tested using high-resolution sector-field

inductively-coupled plasma-mass spectrometry. Mothers with MOM-bearing implants had significantly elevated levels of serum cobalt and chromium compared with control-group mothers, and umbilical cord blood from mothers with MOM implants also had significantly higher serum metal ion levels compared with control-group mothers. The results of this study show that circulating serum levels of metal ion degradation products from MOM bearings cross the placenta and expose the fetus to metal ions. However, the placenta exerts a modulatory effect on cord blood, resulting in decreased levels compared with maternal samples (approximately 15% of maternal chromium and 50% of maternal cobalt). Physicians and women of child-bearing age should be aware of this potential effect when considering the use of MOM-bearing implants.

Maternal exposure to metals--concentrations and predictors of exposure.
Callan A. C., Hinwood A. L., Ramalingam M., Boyce M., Heyworth J., McCafferty P. and Odland J. O.
Environ Res. 2013;126:111-7.

A variety of metals are important for biological function but have also been shown to impact health at elevated concentrations, whereas others have no known biological function. Pregnant women are a vulnerable population and measures to reduce exposure in this group are important. We undertook a study of maternal exposure to the metals, aluminium, arsenic, copper, cobalt, chromium, lithium, manganese, nickel, selenium, tin, uranium and zinc in 173 participants across Western Australia. Each participant provided a whole blood and urine sample, as well as drinking water, residential soil and dust samples and completed a questionnaire. In general the concentrations of metals in all samples were low with the notable exception of uranium (blood U mean 0.07 microg/L, range <0.01-0.25 microg/L; urinary U mean 0.018 microg/g creatinine, range <0.01-0.199 microg/g creatinine). Factors that influenced biological concentrations were consumption of fish which increased urinary arsenic concentrations, hobbies (including mechanics and welding) which increased blood manganese concentrations and iron/folic acid supplement use which was associated with decreased concentrations of aluminium and nickel in urine and manganese in blood. Environmental concentrations of aluminium, copper and lithium were found to influence biological concentrations, but this was not the case for other environmental metals concentrations. Further work is underway to explore the influence of diet on biological metals concentrations in more detail. The high concentrations of uranium require further investigation.

Circulating metals and persistent organic pollutant concentrations in Canadian and non-Canadian born primiparous women from five Canadian centres: results of a pilot biomonitoring study.
Foster W. G., Cheung A. P., Davis K., Graves G., Jarrell J., Leblanc A., Liang C. L., Leech T., Walker M., Weber J. P. and Van O. J.
Sci Total Environ. 2012;435-436:326-36.

The developing foetus is thought to be at increased risk from exposure to environmental contaminants; however, developmental exposure data is notably lacking for many contaminants. Moreover, potential regional differences or effect of place of birth on residue levels measured in pregnant women is also unknown. Therefore, as part of a multinational biomonitoring study, 125 primiparous pregnant Canadian women were recruited from five Canadian centres (Vancouver, Calgary, Hamilton, Ottawa, and Halifax). Metals in whole blood and persistent organic pollutants (POPs) in plasma were measured by inductively coupled plasma mass spectrometry (ICPMS) and gas chromatography-mass spectrometry (GCMS), respectively. Of the 125 women recruited to this study, complete data sets were available for 123 of which 103 were Canadian born. Data were analysed by analysis of covariance and linear mixed models using age and body mass index as covariates. The metals cadmium (Cd), cobalt (Co), lead (Pb), nickel (Ni), selenium (Se), and total mercury (Hg) were detected in more than 93% of the samples tested. β-Hexachlorohexane β-HCH), oxychlordane, trans-nonachlor, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p'-DDE), polybrominated diphenyl ether (PBDE) congeners (PBDE-153, PBDE-47), polychlorinated biphenyl (PCB) congeners (PCB-138, -153, and -180), and the dioxin-like PCB congener PCB-118 were quantified in greater than 70% of the samples tested. Significant differences in the concentrations of Cd, Ni, PCB-153, and p,p'-DDE were found between the centres studied. Furthermore, foreign-born pregnant women had significantly higher concentrations of Cd, β-HCH, PBDE-47, PCB-138, -153, -180, and p,p'-DDE compared to Canadian born pregnant women. Taken together, the data suggest that there are potential regional differences in contaminant body burden and place of birth may also contribute to differences in maternal residue concentrations.

Assessment of essential and nonessential metals and different metal exposure biomarkers in the human placenta in a population from the south of Portugal.
Serafim A., Company R., Lopes B., Rosa J., Cavaco A., Castela G., Castela E., Olea N. and Bebianno M. J.
J Toxicol Environ Health Part A. 2012;75(13-15):867-77.

The general population is exposed to metals as trace amounts of metallic compounds are present in air, water, and food. Information on background exposures and biomarker concentrations of environmental chemicals in the general Portuguese population is limited. Therefore, the purpose of this study was to determine the levels of important nonessential metals with recognized toxicity cadmium (Cd) and lead (Pb) and essential metals copper (Cu), nickel (Ni), chromium (Cr), and zinc (Zn) in placentas of mothers living in south Portugal (Algarve). Due to the difficulty in establishing the effects of chemicals in a complex and variable environment, this study also aimed to examine the response of biomarkers, such as biochemical changes that occurs at subcellular levels in the presence of contaminants. The investigated biomarkers in placentas indicative of metal exposure or damage included the metallothioneins (MT), delta-aminolevulinic acid dehydratase (ALAD) (specific for Pb), and lipid peroxidation (LPO) as an index of oxidative stress damage. Moreover, HJ-BIPLOT was applied in order to identify and categorize mothers vulnerable to environmental contamination in this region. Metal concentrations in the placenta were not excessive but within the range found in most European studies. In general, the biomarkers MT and LPO were positively correlated with metal levels, while with ALAD the opposite occurred, indicating the selected battery of biomarkers were suitable to study the effects of metals on human placenta. Further, the application of multivariate analysis with HJ-BIPLOT showed that most significant factors contributing to maternal and fetal exposures via placenta were dietary and smoking habits.

Inter-rater reliability of assessed prenatal maternal occupational exposures to solvents, polycyclic aromatic hydrocarbons, and heavy metals.
Rocheleau C. M., Lawson C. C., Waters M. A., Hein M. J., Stewart P. A., Correa A., Echeverria D. and Reefhuis J.
J Occ Environ Hyg. 2011;8(12):718-28.

Because direct measurements of past occupational exposures are rarely available in population-based case-control studies, exposure assessment of job histories by multiple expert raters is frequently used; however, the subjective nature of this method makes measuring reliability an important quality control step. We evaluated inter-rater reliability

of 7729 retrospective jobs reported in the National Birth Defects Prevention Study. Jobs were classified as exposed, unexposed, or exposure unknown by two independent industrial hygienists; exposed jobs were further evaluated for intensity, frequency, and routes. Exposure prevalence ranged from 0.1-9.8%. Inter-rater reliability for exposure (yes/no), assessed by kappa coefficients, was fair to good for cadmium (κ = 0.46), chlorinated solvents (κ = 0.59), cobalt (κ = 0.54), glycol ethers (κ = 0.50), nickel compounds (κ = 0.65), oil mists (κ = 0.63), and Stoddard Solvent (κ = 0.55); PAHs (κ = 0.24) and elemental nickel (κ = 0.37) had poor agreement. After a consensus conference resolved disagreements, an additional 4962 jobs were evaluated. Inter-rater reliability improved or stayed the same for cadmium (κ = 0.51), chlorinated solvents (κ = 0.81), oil mists (κ = 0.63), PAHs (κ = 0.52), and Stoddard solvent (κ = 0.92) in the second job set. Inter-rater reliability varied by exposure agent and prevalence, demonstrating the importance of measuring reliability in studies using a multiple expert rater method of exposure assessment.

Maternal and fetal exposure to four carcinogenic environmental metals.
Guan H., Piao F. Y., Li X. W., Li Q. J., Xu L. and Yokoyama K.
Biomed Environ Sci. 2010;23(6):458-65.

OBJECTIVE: To examine maternal and fetal exposure levels to four carcinogenic metals, arsenic (As), cadmium (Cd), nickel (Ni), and beryllium (Be), and to investigate their environmental influences.
METHODS: Metal concentrations in maternal and umbilical cord blood were measured by inductively coupled plasma-mass spectrometry (ICP-MS). Environmental factors that might play a role in exposure were analyzed using Mann-Whitney nonparametric U-tests and multiple linear regression.
RESULTS: The concentrations of As, Cd, and Ni in umbilical cord blood (5.41, 0.87, and 139.54 μg/L) were significantly lower than those in maternal blood (6.91, 1.93, and 165.93 μg/L). There were significant positive correlations between the maternal and cord concentrations of each carcinogen. Our results showed that: (i) exposures to potentially harmful occupational factors during pregnancy were associated with high levels of maternal As, Cd, and Ni; (ii) living close to major transportation routes (< 500 m) or exposure to second-hand smoke during pregnancy increased the maternal Cd levels and (iii) living close to industrial chimneys induced high maternal Ni levels. Multiple linear regression analysis showed that these environmental factors remained significant in models of the influences of these four carcinogens.
CONCLUSION: Both mothers and fetuses had been exposed to As, Cd, Ni, and Be. The increased levels of these carcinogens in pregnant women were associated with

some detrimental environmental factors, such as occupational exposure, contact with second-hand smoke and living close to major transportation routes or industrial chimneys.

Soil metal concentrations and toxicity: associations with distances to industrial facilities and implications for human health.
Aelion C. M., Davis H. T., McDermott S. and Lawson A. B.
Sci Total Environ. 2009;407(7):2216-23.

Urban and rural areas may have different levels of environmental contamination and different potential sources of exposure. Many metals, i.e., arsenic (As), lead (Pb), and mercury (Hg), have well-documented negative neurological effects, and the developing fetus and young children are particularly at risk. Using a database of mother and child pairs, three areas were identified: a rural area with no increased prevalence of mental retardation and developmental delay (MR/DD) (Area A), and a rural area (Area B) and an urban area (Area C) with significantly higher prevalence of MR/DD in children as compared to the state-wide average. Areas were mapped and surface soil samples were collected from nodes of a uniform grid. Samples were analyzed for As, barium (Ba), beryllium (Be), chromium (Cr), copper (Cu), Pb, manganese (Mn), nickel (Ni), and Hg concentrations and for soil toxicity, and correlated to identify potential common sources. ArcGIS was used to determine distances between sample locations and industrial facilities, which were correlated with both metal concentrations and soil toxicity. Results indicated that all metal concentrations (except Be and Hg) in Area C were significantly greater than those in Areas A and B ($p < \text{or} = 0.0001$) and that Area C had fewer correlations between metals suggesting more varied sources of metals than in rural areas. Area C also had a large number of facilities whose distances were significantly correlated with metals, particularly Cr (maximum $r=0.33$; $p=0.0002$), and with soil toxicity (maximum $r=0.25$; $p=0.007$) over a large spatial scale. Arsenic was not associated with distance to any facility and may have a different anthropogenic, or natural source. In contrast to Area C, both rural areas had lower concentrations of metals, lower soil toxicity, and a small number of facilities with significant associations between distance and soil metals.

Metal concentrations in rural topsoil in South Carolina: potential for human health impact.
Aelion C. M., Davis H. T., McDermott S. and Lawson A. B.
Sci Total Environ. 2008;402(2-3):149-56.

Rural areas are often considered to have relatively uncontaminated soils; however few studies have measured metals in surface soil from low population areas. Many metals, i.e., arsenic (As), lead (Pb), and mercury (Hg), have well-documented negative neurological effects, and the developing fetus and young children are particularly at risk. Using a Medicaid database, two areas were identified: one with no increased prevalence of mental retardation and developmental delay (MR/DD) (Strip 1) and one with significantly higher prevalence of MR/DD (Strip 2) in children compared to the state-wide average. These areas were mapped and surface soil samples were collected from 0-5 cm depths from nodes of a uniform grid laid out across the sampling areas. Samples were analyzed for As, barium (Ba), beryllium (Be), chromium (Cr), copper (Cu), Pb, manganese (Mn), nickel (Ni), and Hg. Inverse distance weighting (IDW) was used to estimate concentrations throughout each strip area, and a principal component analysis (PCA) was used to identify common sources. All metal concentrations in Strip 2, the MR/DD cluster area, were significantly greater than those in Strip 1 and similar to those found in more urban and highly agricultural areas. Both Strips 1 and 2 had a high number of significant correlations between metals (33 for Strip 1 and 25 for Strip 2), suggesting possible similar natural or anthropogenic sources which was corroborated by PCA. While exposures were not assessed and direct causation between environmental soil metal concentrations and MR/DD cannot be concluded, the high metal concentrations in areas with an elevated prevalence of MR/DD warrants further consideration.

[The umbilical blood levels of lead and some other toxic metals as a biomarker of environment-induced exposure].
Privalova L. I., Malykh O. L., Matiukhina G. V. and Gnezdilova S. V.
Gig Sanit. 2007;3:68-70.

Groups of pregnant women, which made up in Revda, Pervouralsk, Krasnouralsk, and Verkh-Isetsky District of Yekaterinburg, were studied. Tests of umbilical blood samples (UB) for the levels of calcium, iron, chromium, manganese, zinc, nickel, cadmium, lead, arsenic, copper, and mercury have established that the mean concentration of lead and the proportion of samples with elevated UB lead concentrations depend on how close the residential area is located to the major industrial source of emission of this toxic

metal into ambient air. This correlation is less marked for other metals or it is not found. The particular position of lead is likely to be explained by the fact that it is entirely foreign to an organism and by the comparative unimportance of a contribution of the sources of exposure to this metal, which are unassociated with man-caused environmental and food pollution. As far as other metals are concerned, the situation is complicated by the fact that they are not only toxic, but when upon minor exposures, also essential biotrace elements with controlled and interdependent toxic kinetics. It is also shown that when a pregnant woman takes a complex of biological protectors promoting a reduction in her body's levels of lead, its concentrations in her body, its UB concentration is much lower than such a bioprophylactic effect is absent.

Possible altered mineral metabolism in human anencephalic fetuses.
Friel J. K., Longerich H., Jackson S. E., Pushpananthan C. and Wright J. R., Jr.
Nutr Res. 2005;25(2):103-9.

Neural tube defects are congenital abnormalities caused by failure of the neural tube to close during embryogenesis. We investigated trace elements (zinc, copper, manganese, cobalt, nickel, molybdenum, cadmium), livers, pancreata, sciatic nerves, diaphragms, and kidneys collected at autopsy from 33 anencephalic fetuses and 22 control fetuses. Collections were done on the right side using a titanium scalpel, plastic forceps, and acid-washed materials. Samples were wet ashed and analyzed using inductively coupled plasma mass spectrometry. The gestational age and birth weight (mean ± SD) of anencephalic fetuses were 25.8 ± 8 weeks and 729 ± 879 g, respectively; those of control fetuses, 28 ± 8 weeks and 1340 ± 1216 g, respectively. Liver concentrations (ppm, dry weight, mean ± SEM) of Zn (1075 ± 56 vs 668 ± 75; P = .001) increased in anencephalic fetuses, suggesting defective transport of Zn. Whether this is part of the cause of neural tube defects or a result of the disease is unclear.

Oxytocin inhibits T-type calcium current of human decidual stromal cells.
Liu B., Hill S. J. and Khan R. N.
J Clin Endocrinol Metab. 2005;90(7):4191-7.

CONTEXT: Little is known about the crosstalk between the decidua and myometrium in relation to human labor. The hormone oxytocin (OT) is considered to be a key mediator of uterine contractility during parturition, exerting some of its effects through calcium channels.

OBJECTIVE: The objective was to characterize the effect of OT on the T-type calcium channel in human decidual stromal cells before and after the onset of labor.
DESIGN: The nystatin-perforated patch-clamp technique was used to record inward T-type calcium current (I(Ca(T))) from acutely dispersed decidual stromal cells obtained from women at either elective cesarean section [CS (nonlabor)] or after normal spontaneous vaginal delivery [SVD (labor)].
SETTING: These studies took place at the University of Nottingham Medical School.
RESULTS: I(Ca(T)) of both SVD and CS cells were blocked by nickel (IC(50) of 5.6 microm) and cobalt chloride (1 mm) but unaffected by nifedipine (10 microm). OT (1 nm to 3.5 microm) inhibited I(Ca(T)) of SVD cells in a concentration-dependent manner, with a maximal inhibition of 79.0% compared with 26.2% in decidual cells of the CS group. OT-evoked reduction of I(Ca(T)) was prevented by preincubation with the OT antagonist L371,257 in the SVD but not CS group. OT, in a concentration-dependent manner, displaced the steady-state inactivation curve for I(Ca(T)) to the left in the SVD group with no significant effect on curves of the CS group.
CONCLUSION: Inhibition of I(Ca(T)) by OT in decidual cells obtained during labor may signify important functional remodeling of uterine signaling during this period.

Intercommunity and temporal variation of eleven essential and five toxic elements in human placentas from deliveries in thirteen arctic and sub-arctic areas of Russia and Norway.
Odland J. O., Nieboer E., Romanova N., Hofoss D. and Thomassen Y.
J Environ Monit. 2003;5(1):166-74.

Research is described that constitutes an extension of an earlier paper (J. Environ. Monit., 2001, 3, 177-184), in which concentrations were measured in 263 human placentas of 11 essential elements (P, Ca, Mg, Cu, S, Na, Fe, Zn, K, Se, Mn) and 5 toxic elements (Ba, Sr, Pb, Ni, Cd). The additional data considered derive from earlier visits to 4 of the original 6 communities and 3 others, all but one of which are located in northern Norway and neighbouring areas of Russia. This more than doubled the number of placental samples available (263 to 571). Unfortunately, the personal, life-style and morphometric information obtained for the first study group was not available for the additional mothers. Country differences were evident for all elements except Ba, Fe and Zn; Cd, Cu, Mn, Na, Se, Ni, Pb, Sr and S were higher and K, P, Ca and Mg were lower in Russia ($p < 0.03$). Not unexpectedly, the highest median lead concentration was observed for the largest city in the western arctic region of Russia, namely Murmansk. Similarly, the higher median nickel level observed for Russia reflects the established observation that urinary nickel concentrations are higher in the Russian

than in the Norwegian communities. Even though sampling was performed at different times of the year and before and after a 3-year interval in four centres, inter-collection differences were of relatively small magnitude and appear not to be linked to seasonal or temporal changes. Principal component analysis (PCA) confirmed the prominence of Factor 1, which grouped those metals that are known to form insoluble phosphate complexes and whose concentrations showed a dependence on gestational age and maternal smoking in the earlier study. It is concluded that PCA is a powerful statistical tool for exploring and identifying fundamental pathways and processes involved in governing the inorganic elemental composition of placental tissue. It also has the potential of identifying study limitations and quality assurance shortfalls. Further our findings show promise that placental concentrations of toxic elements may serve as an index of exposure and of nutritional intake for selected essential micro-elements.

Metal ions and human sperm mannose receptors.
Benoff S., Cooper G. W., Centola G. M., Jacob A., Hershlag A. and Hurley I. R.
Andrologia. 2000;32(4-5):317-29.

Zinc and lead concentrations were measured in seminal plasma from fertile donors, infertile men with varicocoele and men undergoing work-ups for in vitro fertilization. Ejaculated spermatozoa from these subjects were incubated in vitro with various metal ions and/or dibromoethane and dibromochloropropane. Mannose receptor expression was correlated with metal and toxicant levels. Sperm distributions of potassium channels were compared with lead ions and calcium channels with zinc ions. Mannose receptor expression by capacitated spermatozoa increased linearly with seminal plasma zinc levels, and correlated inversely with lead levels. Cobalt had no effect on mannose receptor expression, but nickel had a concentration-dependent biphasic effect. Mannose receptor expression was not affected by dibromoethane and dibromochloropropane if the cholesterol content of the sperm membrane was high, but mannose receptor expression was decreased in low cholesterol spermatozoa by exposures below estimated permissive exposure limits. Potassium channels and lead ions co-localized over the entire head of human spermatozoa, while both calcium channels and zinc ions were confined to the equatorial segment of the head. Mannose receptor expression on the external surface of the human sperm plasma membrane is a biomarker for the effects of transition and heavy metals and organic toxicants on sperm fertility potential.

Acrosome reaction of human spermatozoa is mainly mediated by alpha1H T-type calcium channels.
Son W. Y., Lee J. H., Lee J. H. and Han C. T.
Mol Hum Reprod. 2000;6(10):893-7.

The objectives of this study were: (i) to investigate the possible role of T-type Ca(2+) channels on the acrosome reaction (AR) of human spermatozoa; and (ii) to determine the sub-type of T-type calcium channels involved in the AR. The AR was induced in vitro by mannose-bovine serum albumin (BSA). The inhibitory effects of mibefradil (T-type Ca(2+) channel blocker), NiCl(2), or nifedipine (L-type Ca(2+) channel blocker) on the mannose-BSA induced AR were evaluated in capacitated human spermatozoa. The AR was sensitively inhibited by low micromolar concentrations of mibefradil (IC(50) = 1 micromol/l) in a dose-dependent manner. Low concentrations of Ni(2+) (IC(50) = 40 micromol/l) also inhibited the mannose-BSA induced AR. On the contrary, higher concentrations of nifedipine were required to block AR (IC(50) = 60 micromol/l). Reverse transcription-polymerase chain reaction (RT-PCR) was performed to identify the sub-types of T-type channels present in human testes. Analysis of PCR products showed that only alpha1H subunits are expressed in testes. The expression of the alpha1H subunit may be tissue specific since its mRNA was not detected in the human ovary. The present study suggests that the AR of human spermatozoa is highly associated with T-type Ca(2+) channels and is mainly mediated by calcium influx through alpha1H T-type Ca(2+) channels.

Nickel toxicity to human term placenta: in vitro study on lipid peroxidation.
Chen C. Y. and Lin T. H.
J Toxicol Environ Health Part A. 1998;54(1):37-47.

It has been reported that nickel (Ni) crosses the human placenta and produces teratogenesis and embryotoxicity. In the present study, the effects of nickel on human term placentas were investigated. In time-course experiments, placental tissue was incubated for 3, 6, 12, or 24 h with 2.5 mM Ni. The viability as determined by glucose consumption rate did not show any significant change from 3 to 12 h, whereas the permeability, lipid peroxidation, and Ni concentration were significantly increased compared to the control. In concentration-response studies, placental explants were incubated with 0.5, 1.0, 2.5, or 5 mM Ni for 12 h. The viability did not change significantly, except for 5 mM Ni, but the permeability and lipid peroxidation increased markedly in a concentration-dependent manner. Treatment with ascorbic acid or Zn decreased placental lipid peroxidation and permeability induced by Ni, but had no effect

on lowering the Ni tissue content. Data show that Ni is toxic as evidenced by lipid peroxidative damage to placental membrane, and this metabolic change may be responsible for decreased placental viability, altered permeability, and potential subsequent embryotoxicity.

Sites of lead and nickel accumulation in the placental tissue.
Reichrtova E., Dorociak F. and Palkovicova L.
Hum Exp Toxicol. 1998;17(3):176-81.

1. There is a variety of quantitative analytical data regarding the total concentrations of heavy metals in human placentae. However, little is known about sites of metal accumulation in the placental tissue structural zones in relation to the environment. In this study, the lead and the nickel particulate deposits in the placental chorionic plate, the chorionic villous tree and the basal plate, using tissue histochemical reactions for lead and nickel, have been estimated. The degree of metal contamination of placentae was assessed according to an arbitrary scale. Both metals have shown a common mode of accumulation in the placental tissue structural zones. Among the observed contaminated structures in the placental tissue, the syncytiotrophoblast was the most frequent site for lead and nickel particulate deposits. 2. The frequency distribution of both metals within the mentioned placental zones, using four metal contamination degrees, was determined. A heavily contaminated zone was found to be the chorionic villous tree, especially in samples from the industrial region. 3. A comparison between two Slovak regions (an industrial and a rural one) using statistical tests was performed. The frequency of samples without any lead occurrence in the chorionic villous tree was found to be 10% in the industrial region, and 16% in the rural region. Regarding the nickel deposits, the frequency of non-contaminated samples in the industrial region was 2%, whereas in the rural region 6%. A relationship between traffic related pollution and human placenta contamination was found in both investigated regions.

Prenatal exposure to heavy metals: effect on childhood cognitive skills and health status.
Lewis M., Worobey J., Ramsay D. S. and McCormack M. K.
Pediatrics. 1992;89(6 Pt 1):1010-5.

Prenatal exposure to seven heavy metals (cadmium, chromium, cobalt, lead, mercury, nickel, and silver) was determined for amniotic fluid taken from 92 pregnant women undergoing amniocentesis at approximately 16 to 18 weeks' gestation. Follow-up assessment of their children's cognitive skills and health status was conducted when the

children were approximately 3 years of age. The presence of these metals co-occurred in amniotic fluid. A prenatal toxic risk score was derived which was a weighted score reflecting the presence of the various metals in amniotic fluid. The toxic risk score was negatively related to performance on the McCarthy Scales of Children's Abilities and positively related to the number of child illnesses reported. These results suggest the need for further prospective research on the adverse effects of prenatal exposure to various metals in combination.

[10-year comparison of deposits of heavy metals in the human placenta].
Thieme R., Schramel P. and Keiler G.
Geburtshilfe Frauenheilkund. 1986;46(3):180-4.

The environmental influence on the heavy metal's (trace elements) content of the human placenta was determined in tissues from three different regions of the FRG by different analytical techniques. Besides the mean values and the standard deviation the frequency distribution curves of the elements cadmium, lead, zinc, copper, cobalt, manganese and nickel are given. Comparing the results with our findings from ten years ago it seems that cadmium concentrations in the placenta have reached an approximately equal level both in rural and in industrial districts. This is equally true for lead.

Copper, manganese, zinc, nickel, cadmium and lead in human foetal tissues.
Casey C. E. and Robinson M. F.
Br J Nutr. 1978;39(3):639-46.

1. Concentrations of copper, manganese, zinc, nickel, cadmium and lead were measured in samples of liver, kidney, brain, heart, lung, skeletal muscle and vertebral bone from forty foetuses of 23-43 weeks gestation. 2. Cu concentrations in the liver were up to 100 times those in other tissues, but only those in the brain showed a significant increase with gestational age. 3. Mn concentrations were similar in all tissues; the over-all range was 0.35-9.27 microgram/g dry matter (DM). 4. Concentrations of Zn in the liver were much higher than in other tissues and decreased with gestational age, whereas levels in skeletal muscle increased. 5. In all tissues Ni concentrations were within the range 0.04-2.8 microgram/g DM and levels in kidney and muscle decreased significantly with age. 6. Cd was detected in most of the tissue samples and concentrations were within the range 0.01-0.58 microgram/g DM. 7. Concentrations of Pb, where it was detected, varied from 0.1 to 2.4 microgram/g DM in the soft tissues and from 0.4 to 4.3 microgram/g DM in the bone samples.

E. Titles only (abstracts not available)

Reduction in male births among workers exposed to metal fumes.
Figa-Talamanca I. and Petrelli G.
Int J Epidemiol. 2000;29(2):381.

Cytotoxic effects of welding fumes on human embryonic epithelial pulmonary cells in culture.
Hildebrand H. F., Collyn-Dhooghe M. and Stern R. M.
Excerpta Med Int Congr Ser. 1986;676:319-24.

Alterations of human fetal kidney epithelial cells produced by nickel sulfate.
Haugen A., Hansteen I. L. and Dalen H.
Nickel Toxicol,. 1985.

[Embryotoxic effect of nickel entering the body via drinking water].
Nadeenko V. G., Lenchenko V. G., Arkhipenko T. A., Sa, chenko S. P. and Petrova N. N.
Gig Sanit. 1979;6:86-8.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats.
Kong L., Tang M., Zhang T., Wang D., Hu K., Lu W., Wei C., Liang G. and Pu Y.
Int J Mol Sci. 2014;15(11):21253-69.

Nickel is associated with reproductive toxicity. However, the reproductive toxicity of nickel nanoparticles (Ni NPs) is unclear. Our goal was to determine the association between nickel nanoparticle exposure and reproductive toxicity. According to the one-generation reproductive toxicity standard, rats were exposed to nickel nanoparticles by gavage and we selected indicators including sex hormone levels, sperm motility, histopathology, and reproductive outcome etc. Experimental results showed nickel nanoparticles increased follicle stimulating hormone (FSH) and luteinizing hormone (LH), and lowered etradiol (E2) serum levels at a dose of 15 and 45 mg/kg in female

rats. Ovarian lymphocytosis, vascular dilatation and congestion, inflammatory cell infiltration, and increase in apoptotic cells were found in ovary tissues in exposure groups. For male rats, the weights decreased gradually, the ratio of epididymis weight over body weight increased, the motility of rat sperm changed, and the levels of FSH and testosterone (T) diminished. Pathological results showed the shedding of epithelial cells of raw seminiferous tubule, disordered arrangement of cells in the tube, and the appearance of cell apoptosis and death in the exposure group. At the same time, Ni NPs resulted in a change of the reproductive index and the offspring development of rats. Further research is needed to elucidate exposure to human populations and mechanism of actions.

The toxic effects of nickel chloride on liver, erythropoiesis, and development in Wistar albino preimplanted rats can be reversed with selenium pretreatment.
Adjroud O.
Environ Toxicol. 2013;28(5):290-8.

The exposure to nickel chloride (NiCl2) can cause hematotoxicity and hepatotoxicity and can affect development. The present study pertains to the protective effect of selenium (Se) against NiCl2-induced toxicity in preimplanted Wistar albino rats. The subcutaneous (s.c.) administration of 25 or 50 mg/kg of NiCl2 to Wistar albino rats on day 3 of gestation induced an immediate and significant decrease in maternal body weight and anemia 2 days after treatment. In addition, an increase in plasma aspartate aminotransferase (AST) was observed. These effects were maintained on day 20 of gestation. Moreover, a significant increase in plasma alanine aminotransferase (ALT) levels was observed with the administration of 25 mg/kg of NiCl2. Conversely, administration of 50 mg/kg of NiCl2 by s.c. injection increased erythropoiesis at day 20 of gestation and decreased platelets counts. In addition, administration of 100 mg/kg of NiCl2 markedly reduced the maternal body weight and number of live fetuses and increased fetal loss, predominantly at the end of the experimental period. All dose levels of NiCl2 caused an alteration in the hepatic histoarchitecture. When 0.3-mg/kg Se was injected s.c. with 100-mg/kg NiCl2, the levels of plasma AST and ALT and the structure of the liver were restored. Administration of 20 mg/L/day of NiCl2 in the drinking water significantly reduced the maternal body weight at day five of gestation as well as erythropoiesis during the exposure period. The present study suggests that Se can counteract the nocuous effect of nickel on the liver; however this antioxidant did not prevent alterations in development and erythropoiesis.

Embryotoxic and teratogenic effects of nickel in Swiss albino mice during organogenetic period.
Saini S., Nair N. and Saini M. R.
Biomed Res Int. 2013;2013:701439.

The present study evaluates potential hazardous of nickel (Ni(+2) as NiCl2·6H2O) to Swiss albino mice fetus. Ni was administered orally on body weight base from days 6 to 13 of gestation period. Based on LD50, Ni doses (46.125, 92.25, and 184.5) mg Ni/kg b.wt. were used. On day 18 of gestation, uteri of the sacrificed dams were examined. A dose-dependent decrease ($P < 0.01$) in the body weight of the pregnant females and fetuses during the gestation period was observed. Number of implant sites and placental weight at all the three dose levels was lower compared with their respective control groups. Average number of live fetuses/dams reduced significantly ($P < 0.01$) at 184.5 mg Ni/kg b.wt. with concomitant increase in the percentage of postimplantation death and percentage of resorbed, macerated, and dead fetuses, respectively. Exposure increased the fetal malformations, namely, hydrocephaly, open eyelids, microphthalmia, exophthalmia, club foot, umbilical hernia, and skeletal anomalies. Reduced ossification of nasal, frontal, parietal, intraparietal, and supraoccipital bones, absence/gap between the ribs, reduced/fused sternebrae, vertebral centra, and caudal vertebrae, reduced pelvic elements, absence of carpals, metacarpals, tarsals, metatarsals, and phalanges were distinct. This indicates vulnerability of the mice fetus to nickel during prenatal exposure.

α-Tocopherol ameliorates nickel induced testicular oxidative and nitrosative stress in albino rats.
Jargar J. G., Yendigeri S. M., Hattiwale S. H., Dhundasi S. A. and Das K. K.
J Basic Clin Physiol Pharmacol. 2012;23(2):77-82.

BACKGROUND: Heavy metals generate free radicals and induce oxidative and nitrosative stress with depletion of antioxidants. In this study, we have evaluated the beneficial effects of α-tocopherol against nickel sulfate exposed testicular dysfunction.
METHODS: We studied the effect of supplementation of α-tocopherol (10 mg/100 g body weight, i.m.) on nickel sulfate (2.0 mg/100 g body weight, i.p.) induced testicular oxidative and nitrosative stress in Wister strain male albino rats. Serum and testicular nitric oxide, L-ascorbic acid and serum α-tocopherol concentrations were evaluated. We also evaluated sperm count, motility and histopathology of testes.
RESULTS: Nickel treated rats showed significantly decreased body weight, testicular somatic index, sperm count, sperm motility, serum and testicular L-ascorbic acid

concentration and serum α-tocopherol level as compared to their controls. However, simultaneous treatment with nickel sulfate and α-tocopherol produced a remarkable improvement of all the above parameters when compared with treatment with nickel alone. Nickel treated rats also had significantly increased serum and testicular nitric oxide concentrations as compared to their controls. However, simultaneous treatment with nickel sulfate and α-tocopherol significantly decreased nitric oxide concentrations in both serum and testes, respectively, as compared to nickel treatment alone. Histopathology of the testes revealed tortuous seminiferous tubules, loss of spermatogenesis process (> 75%), congestion and necrosis in nickel sulfate treated rats, whereas rats simultaneously treated with nickel sulfate and α-tocopherol had almost normal seminiferous tubules and near normal spermatogenesis as compared to nickel alone treated rats.
CONCLUSIONS: Nickel sulfate treatment causes testicular oxidative and nitrosative stress in albino rats, but simultaneous supplementation of α-tocopherol was found to be beneficial in combating against such stresses.

Quantitative histological analysis of the mouse testis after the long-term administration of nickel in feed.
Toman R., Mass nyi P., Adamkovicova M., Lukac N., Cabaj M. and Martiniakova M.
J Environ Sci Health Part A Tox Hazard Subst Environ Eng. 2012;47(9):1272-9.

In this study, the effects of nickel chloride (NiCl(2)) applied per os on testis histopathology and morphometry of mice were investigated. The metal was applied in pellets at a dose of 10 mg NiCl(2)/kg bw to male mice 4 weeks of age. After 3, 6, 9 and 12 weeks of nickel administration, the relative volume of whole seminiferous tubule, germinal epithelium, tubule lumen, interstitium and blood vessels as well as the diameter of seminiferous tubules were determined in the experimental and corresponding control groups. Microscopic examination of testis showed significant changes in all nickel-exposed groups. The degeneration of germinal epithelium, with released germ cells into the lumen of the tubules, and occurrence of empty spaces in the seminiferous epithelium were found in all experimental groups. The changes in the testes were time-dependent. The relative volume of empty spaces in the seminiferous epithelium significantly increased ($P < 0.001$) in all experimental groups when compared with the corresponding control. A significant decrease in the relative volume of seminiferous epithelium was observed after 6 and 12 weeks of Ni-exposure. The increased luminization of the tubules was found after 6 ($P < 0.001$), 9 ($P < 0.01$) and 12 ($P < 0.001$) weeks. Interstitial tissue significantly decreased after 6 and 9 weeks of Ni exposure and increased after 12 weeks of Ni intake. The seminiferous tubule diameter

significantly (P < 0.001) decreased after 12 weeks. Results of this study report a serious, time-dependent changes in the testes, mainly in the germinal epithelium, after a peroral intake of nickel.

Oxidative stress level in the testes of mice and rats during nickel intoxication.
Murawska-Cialowicz E., Bal W., Januszewska L., Zawadzki M., Rychel J., Zuwala-Jagiello J.
The Sci World J. 2012;2012:395741.

The genotioxic and carcinogenic effect of nickel probably results from its capacity to produce reactive oxygen species (ROS) and disturb the redox balance. The aim of the study was to find out if rats lacking spermatic protamine 2 are less susceptible to Ni(II) than mice. Consequently, the levels of malondialdehyde + 4 hydroxynonenal (MDA+4HDA) - markers of lipid peroxidation, as well as the level of reduced glutathione (GSH) were measured within the rat and mouse testes. Our results showed that the levels of lipid peroxidation markers were elevated in testicular homogenates of intoxicated mice without any changes in rats. GSH level was lower in the group of intoxicated mice comparing to the control without statistically significant changes in rats' homogenates. Moreover, the level of GSH in the testes of intoxicated mice was lower than in rats. On the basis of our results, it appears that Ni(II) can initiate oxidative stress in the testes of mice but not of rats and can reduce GSH level. Consequently, the antioxidative defense of the testes is reduced. Ni(II) that causes oxidative stress in the testes may also contribute to infertility.

Protective effects of grape seed procyanidin extract against nickel sulfate-induced apoptosis and oxidative stress in rat testes.
Su L., Deng Y., Zhang Y., Li C., Zhang R., Sun Y., Zhang K., Li J. and Yao S.
Toxicol Mech Methods. 2011;21(6):487-94.

This study determined whether nickel sulfate (Ni)-induced reproductive damage occurs via apoptosis and oxidative stress and to examine the expression of Bax and c-kit and their effects on Ni exposure. The study also explored the protective effects of grape seed proanthocyanidin extract (GSPE) against Ni toxicity in the testes. Wistar rats were treated with normal saline, Ni alone (1.25, 2.5, and 5 mg/kg/day), and Ni (2.5 mg/kg/day) plus GSPE (50 and 100 mg/kg/day). After 30 days, Ni significantly decreased sperm motility and the percentage of S-phase cells and enhanced testicular apoptosis in the 2.5 and 5 mg groups. The levels of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO) significantly increased. The decreased activity of glutathione

peroxidase and catalase in the Ni groups showed that Ni could increase oxidative stress, especially at 2.5 and 5 mg. Western blot analysis showed that the expression of Bax protein and c-kit increased in 2.5 and 5 mg Ni groups compared with controls. Conversely, these changes were partially attenuated in rats simultaneously administered GSPE, especially in the 100 mg group. These results demonstrate the following: (1) Ni exhibits reproductive toxicity in rats by decreasing sperm at concentrations of 2.5 and 5 mg; (2) intratesticular apoptosis, oxidative stress, and c-kit overexpression play pivotal roles in reproductive damage induced by Ni; and (3) GSPE enhances sperm motility by down-regulating c-kit expression and offsetting the apoptosis and oxidative stress induced by Ni by directly decreasing MDA and NO, scavenging H_2O_2, and down-regulating Bax expression.

Embryo development, stress protein (Hsp70) responses, and histopathology in zebrafish (Danio rerio) following exposure to nickel chloride, chlorpyrifos, and binary mixtures of them.
Scheil V., Zürn A., Köhler H. R. and Triebskorn R.
Environ Toxicol. 2010;25(1):83-93.

Two different classes of chemicals were tested in a multilevel approach in this study: NiCl(2) as a representative for heavy metals and chlorpyrifos, a pesticide. Both, the single substances and mixtures of them were investigated for their effects on embryonic development, histological alterations, and the stress protein (Hsp70) response in the zebrafish Danio rerio. Fishes were exposed from fertilization of eggs up to a maximum of 168 h post fertilization, depending on the investigated endpoint. NiCl(2) led to effects in all tests which, however, were less severe at the histopathological level than in developmental (hatching success) and stress protein studies. Chlorpyrifos did not lead to developmental alterations but it was found to induce the Hsp70 response as well as histopathological damages. Mixtures of both substances resulted in similar results as the single substances; the results suggest an independent mode of action of these two substances and additivity of their effects.

Toxicity and developmental defects of different sizes and shape nickel nanoparticles in zebrafish.
Ispas C., Andreescu D., Patel A., Goia D. V., Andreescu S. and Wallace K. N.
Environ Sci Tech. 2009;43(16):6349-56.

Metallic nanoparticles such as nickel are used in catalytic sensing, and electronic applications, but health and environmental affects have not been fully investigated.

While some metal nanoparticles result in toxicity, it is also important to determine whether nanoparticles of the same metal but of different size and shape changes toxicity. Three different size nickel nanoparticle (Ni NPs) of 30, 60, and 100 nm and larger particle clusters of aggregated 60 nm entities with a dendritic structure were synthesized and exposed to zebrafish embryos assessing mortality and developmental defects. Ni NPs exposure was compared to soluble nickel salts. All three 30, 60, and 100 nm Ni NPs are equal to or less toxic than soluble nickel while dendritic clusters were more toxic. With each Ni NP exposure, thinning of the intestinal epithelium first occurs around the LD10 continuing into the LD50. LD50 exposure also results in skeletal muscle fiber separation. Exposure to soluble nickel does not cause intestinal defects while skeletal muscle separation occurs at concentrations well over LD50. These results suggest that configuration of nanoparticles may affect toxicity more than size and defects from Ni NPs exposure occur by different biological mechanisms than soluble nickel.

Influence of nickel chloride, chlorpyrifos, and imidacloprid in combination with different temperatures on the embryogenesis of the zebrafish Danio rerio.
Scheil V. and Köhler H. R.
Arch Environ Contam Toxicol. 2009;56(2):238-43.

Two independent types of stressors, chemicals and high temperatures, which frequently act together in the environment, are addressed in this study. Pesticides (imidacloprid and chlorpyrifos) as well as a metal salt (nickel chloride) were investigated for their toxic effect at different temperatures. Tests focused on the early development of zebrafish (Danio rerio) embryos and larvae (from fertilization up to 168 h postfertilization) when exposed to the three respective chemicals at an optimum temperature (26 degrees C) and three higher temperatures (up to 33.5 degrees C). At all temperatures tested, the two pesticides did not have a significant impact on the early development of the zebrafish at the highest test concentrations (imidacloprid, 50 mg/l; chlorpyrifos, 1 mg/l). Nickel led to a significant decrease of hatching success at all temperatures; the combination of elevated temperature and nickel exposure revealed a synergistic effect of both stressors.

Protective role of vitamin E on nickel and/or chromium induced oxidative stress in the mouse ovary.
Rao M. V., Chawla S. L. and Sharma S. R.
Food Chem Toxicol. 2009;47(6):1368-71.

In the present study, we report the invivo effects of nickel chloride (NiCl2; 8 and 16 mg/kg body weight) and/or potassium dichromate (K2Cr2O7; 5 and 10mg/kg body weight) in the ovary of adult mice. The protective role of vitamin E (2mg/kg body weight) along with their combination was also studied. Nickel and/or chromium to mice enhanced the levels of lipid peroxides in the ovary, which was accompanied by a significant decline in the levels of protein, glutathione, total ascorbic acid and activities of superoxide dismutase and catalase. Supplementation of vitamin E along with NiCl2 + K2Cr2O7 significantly lowered the levels of lipid peroxidation and enhanced the antioxidant status. Findings of the present study suggest that vitamin E exerts its protective effect against nickel and/or chromium induced toxicity by preventing lipid peroxidation and protecting antioxidant system in the mouse ovary.

Effect of l-ascorbic acid on antioxidant defense system in testes of albino rats exposed to nickel sulfate.
Gupta A. D., Dhundasi S. A., Ambekar J. G. and Das K. K.
J Basic Clin Physiol Pharmacol. 2007;18(4):255-66.

We studied the effect of oral supplementation with L-ascorbic acid (50 mg/100 g body weight) on nickel sulfate (2.0 mg/100 g body weight, i.p.) induced lipid peroxidation in the testes of Wister strain male albino rats. Testicular lipid peroxide and glutathione (GSH) levels and the activities of the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were estimated. Nickel sulfate treatment significantly increased the level of testicular lipid peroxide and decreased all antioxidant enzymes activities and GSH concentration. Simultaneously treatment of L-ascorbic acid exhibited a possible protective role on the toxic effect of nickel sulfate on testicular lipid peroxide and GSH concentration as well as antioxidant enzymatic defense system.

[Impairment effect of nickel sulfate on ovarian cells in female mice].
Wang X. H. and Zhu Y. Z.
Chung Kuo Kung Kung Wei Sheng. 2007;23(2):212-3.

Objective: To study the toxic effect and its mechanism of apoptosis induced by nickel sulfate in ovarian cells of female mice, and to offer scientific basis for preventing and curing premature ovarian failure (POF). Methods: Healthy female kunming mice were injected inraperitoneally with different doses of nickel sulfate (10.0, 5.0, 2.5 mg/kg) continuously for 12 days. Vaginal smears of mice were taken to determine estrous cycle. Morphological changes of apoptotic cells were observed by light microscopy, and the cell cycle and DNA content were analyzed by flow cytometry (FCM). Results: (1) The dioestrus was prolonged and irregular in toxicant exposure groups. (2) By light microscopy, there were many morphological characteristics of apoptosis including compaction and margination of nuclear chromatin, nuclear fragments, and apoptotic bodies. (3) The results of FCM showed that nickel sulfate could result in the arrest of G0/G1 phase and reduce ther percentage of G2/M S phase. Ther percentage of apoptosis and D.I was also increased while PI was reduced. Conclusion: Intraperitoneal-injected nickel sulfate could induce the lesion of mice's ovary.

[Effects of Ni2+ and Cr 6+ on vimentin of testis in adult rats].
Ren J. H. and Zhu W. J.
Shengzhi Yu Biyun. 2007;27(6):378-81.

Objective: To investigate the expression of vimentin in adult rat testis after combination of nickel ion (Ni2+) and hexavalent chromium (Cr6+) exposure. Method: A total of 32 male SD rats were divided into 8 groups randomly. The groups were group A (control group), group B (0.9% NaCl), group C (5 mg/kg Ni2+), group D (50 mg/kg Ni2+), group E (2.5 mg/kg Cr 6+), group F (6.5 mg/kg Cr6+), group G (5 mg/kg Ni2+ + 2.5 mg/kg Cr6+), and group H (50 mg/kg Ni2+ + 6.5 mg/kg Cr6+). Animals in groups B-H were administrated by gastric perfusion for 4 months. The expression of vimentin in testis was detected by immunohistochemistry method. Results: Compared with control, the expression of vimentin in Sertoli cells was significantly decreased in groups C-H ($P < 0.01$), and group G had more decrease than group C or E ($P < 0.01$), and group H and D or F ($P < 0.01$). In testicular interstitium, the expression of vimentin in groups C-H had significant decrease compared with control group ($P < 0.01$), and group G had more decrease than group C or E ($P < 0.01$), and group H and D or F ($P < 0.01$). Conclusion: Expression of vimentin of Sertoli cells and testicular interstitium in male rat would be

significantly decreased after Ni2+ or Cr 6+ exposure. The combination of Ni2+ and Cr6+ could cause more damage to testis.

Nickel-induced oxidative stress in testis of mice: evidence of DNA damage and genotoxic effects.
Doreswamy K., Shrilatha B., Rajeshkumar T. and Muralidhara
J Androl. 2004;25(6):996-1003.

Oxidative stress (OS) mechanisms are speculated to play a significant role in nickel-induced toxic effects and their carcinogenic potency. Although nickel-induced oxidative damage in somatic tissues is well demonstrated, evidence of the involvement of a similar mechanism(s) in nickel-induced testicular dysfunction and associated genotoxic effects is scarce. Hence, the present study aimed to investigate the nickel-induced OS response in testis and the associated genotoxic implications in vivo. Initially, the toxicity profile of nickel chloride was determined in adult albino mice (CFT-Swiss) following administration (intraperitoneal) of single doses. Subsequently, multiple sublethal doses (1.25, 2.5, and 5.0 micromol/100 g of body weight per day for 3 days) were used to characterize effects on testicular histoarchitecture, lipid peroxidation (LPO) in testis (homogenates, microsomal or mitochondrial fractions) and epididymal sperm, DNA damage, induction of apoptosis in testis, and incidence of sperm head abnormalities. Although short-term doses of nickel induced only a minimal LPO response, multiple doses elicited a moderate (15% to 30%) increase in LPO in whole homogenates and higher dose-related increases in both mitochondrial (20% to 50%) and microsomal fractions (25% to 60%). This was associated with a significant increase in DNA damage in the testis as evidenced by increased single-strand breaks (fluorimetric analysis of DNA unwinding assay). Further, at higher doses, nickel-induced apoptosis was demonstrable in the testis biochemically. Although caudal sperm counts determined at all sampling weeks showed no alterations, analysis for head abnormalities revealed a nearly 3- to 4-fold increase in the percentage of abnormal sperms among the nickel-treated males during the first 3 weeks. Furthermore, mating of nickel-treated (2.5 micromol/100 g of body weight per day for 5 days) males sequentially for a period of 5 weeks with untreated females resulted in a significant increase in male-mediated dominant lethal-type mutations (the frequency of dead implantations) during the first 3 weeks, suggesting a stage-specific effect on postmeiotic germ cells. These findings suggest that testicular toxicity of nickel compounds may be related to enhanced production of reactive oxygen species, probably mediated through oxidative damage to macromolecules, including damage to DNA.

[Toxic effect of nickel sulfate on germ cells of rats and NOS].
Wang X. X. and Sun Y. B.
Chung Kuo Kung Kung Wei Sheng. 2004;20(7):830-1.

Objective: To study the toxic effect of nickel sulfate on the germ cells in female rats and its mechanism. Methods: Nickel sulfate was injected intraperitoneally for Wistar female rats with sexual maturation daily at different doses (1.25, 2.50, 5.00 mg/kg) for 21 days. The number of superovulation and surviving ovum were observed through cell culture. Activity of nitric oxide synthase (NOS) and nitric oxide (NO) content in the ovary were measured. Results: The number of superovulation and surviving ovum was lower than those in the control group; activity of NOS and NO content in the ovary of the rats given nickel sulfate were higher compared with control group. Conclusion: The ovary of the female rats was damaged by peritoneal injection of nickel sulfate, the number of superovulation and surviving ovum decrease was possibly associated with the increase of activity of NOS and NO content in the ovary.

[Effect of nickel sulfate on gonad of female rats].
Wang X. X. and Zhu Y. Z.
Chung Kuo Kung Kung Wei Sheng. 2003;19(8):946-7.

Objective: To study the toxic effect of nickel sulfate on hypothalamic-pituitary-ovarian axis of female rats. Methods: Wistar female rats were given by abdomen with different doses of nickel sulfate (5.00, 2.50, 1.25 mg/kg) for 21 days and the estrus cycle of rats was observed, the changes of Ni levels in vagina, uterus, ovary and hypophysis were measured, respectively. The GnRH-stimulating test were carried in rats, with the serum FSH, LH, P and E2 determined before the injection of GnRH and the serum FSH, LH determined after the injection of GnRH finally the super-ovulation test was done. Results: The Ni contents in the vagina, uterus and ovary of the 5.00, 2.50 mg/kg rats were higher than in the control ($P < 0.01$). The Ni contents in the uterus and ovary of the 1.25 mg/kg rats were higher than in the control ($P < 0.05$); before the injection of GnRH, the levels of FSH and LH were higher than in the control ($P < 0.01$), but there were no significant difference of serum FSH and LH between groups after the injection of GnRH; The levels of serum E2, P of the 5.00, 2.50 mg/kg rats were lower than in the control ($P < 0.05$ or $P < 0. 01$); There was significant difference of the amount of ova between the 5.00 mg/kg group and the control group in the super - ovulation test. Conclusion: The ovary of the female rats were damaged by nickel sulfate given by abdomen.

Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction.
Das K. K. and Dasgupta S.
Environ Health Perspect. 2002;110(9):923-6.

Nickel, a widely used heavy metal, exerts potent toxic effects on peripheral tissues as well as on the reproductive system. Low dietary protein coupled with exposure to this metal induces more severe changes, including biochemical defects, structural disorders, and altered physiologic functions. This study was designed to assess the effects of nickel sulfate on testicular steroidogenesis and to ascertain whether such alterations are reversible with normal protein and protein-restricted dietary regime. Nickel sulfate [2 mg/100 g body weight (bw)] dissolved in double-distilled water was administered on alternate days for 10 doses in a normal protein diet (18% casein) and a protein-restricted diet (5% casein) to Wistar male albino rats (bw 160 +/- 5 g). Two groups, one with a normal protein diet and the other with a protein-restricted diet, served as controls. Twenty-four hours after the last treatment, all the animals except those in withdrawal groups were sacrificed by decapitation. We observed a significant reduction in the activities of the testicular steroidogenic enzymes and plasma testosterone concentration accompanied by a significant elevation in cholesterol and ascorbic acid level in both dietary groups. After 15 days of withdrawal from the nickel sulfate treatment, the testicular steroidogenic enzymes, along with plasma testosterone level, improved significantly in both normal protein-fed and protein-restricted dietary groups. The effects of nickel on testicular cholesterol and ascorbic acid concentration were also reduced after withdrawal. Our results indicate that nickel sulfate affects the steroidogenic enzymes, causing alteration in the formation of testosterone in both dietary groups, which was manifested in the elevated cholesterol and ascorbic acid level with decreased activities of steroidogenic enzymes in adult rats testes. However, these alterations were reversible in both groups of animals fed normal protein diets and protein-restricted diets.

Effect of nickel on testicular nucleic acid concentrations of rats on protein restriction.
Das K. K. and Dasgupta S.
Biol Trace Elem Res. 2000;73(2):175-80.

The nucleic acids (DNA and RNA) and total protein concentration in testes were estimated in male Wistar strain rats treated intraperitorally with nickel sulfate (2.0 mg/100 g body weight) on alternate days for 10 dosages. In both normal (18% casein)

and protein-restricted (5% casein) experimental animals, the nucleic acids and total protein concentration were found to decrease significantly compared to the corresponding controls. Sperm count and sperm motility were also reduced in both experimental groups of animals. The results indicate that nickel influences the expression of genetic information by reducing testicular nucleic acids and protein concentration in both dietary experimental groups.

Effects of nickel chloride on reproduction of the rat and possible antagonistic role of selenium.
Käkelä R, Käkelä A, Hyvärinen H.
Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1999;123(1):27-37.

Nickel (10-100 ppm added as NiCl2) was studied to determine its effects on reproduction of Wistar rats. In nine experimental groups, females, males or both were exposed to nickel in drinking water. In one female group and one male group, the drinking water was also supplemented with 0.3 ppm selenium (added as Na2SeO3). Breeding success and the growth and viability of pups were recorded. Nickel, copper and zinc concentrations in kidneys, liver and skin (with fur) of the females, males and pups were determined with an atomic absorption spectrophotometer. In addition, histology of the male testes (from control and nickel-exposed groups) was studied. The female exposures started 14, 28 or 100 days before copulation and continued during pregnancy and lactation. When the males were exposed (for 28 or 42 days before copulation), NiCl2 reduced both the number of pregnancies and the number of pups born. In the testes, NiCl2 induced shrinkage of the seminiferous tubules, which seemed to close some of the tubules. In the tubules, NiCl2 decreased the number of basal spermatogonia. When the females or both parents were exposed to NiCl2, pup mortality during lactation was high. However, when the females were drinking NiCl2 supplemented with selenium, all the pups survived and development of the total mass of the litters was even better than in the control group. In the same way, in males, selenium supplementation of the drinking water protected those pups that were born; but fertility was lower than with the control treatment. In the tissues studied, nickel accumulated most in the kidneys and then in the liver and skin. In each type of organ, there was a clear dose response relationship. In the pups, in particular, selenium (given to the females) increased the amount of nickel in tissues compared with corresponding administration of nickel without selenium. In summary, selenium seemed to counteract the deleterious effects of NiCl2 on the reproduction of rats.

Male reproductive effect of nickel sulphate in mice.
Pandey R., Kumar R., Singh S. P., Saxena D. K. and Srivastava S. P.
Biometals. 1999;12(4):339-46.

Nickel sulphate was administered orally to adult male mice at dose level of 5 and 10 mg/kg body weight (5 days per week) for 35 days. There was no change in body weight. However a significant decrease in absolute and organ-to-body weight ratios of testes, epididymides, seminal vesicles and prostate gland was observed. The sperm abnormality, associated with decrease in sperm motility and sperm count was also observed. Significant alterations in the activities of marker testicular enzymes, viz. sorbitol dehydrogenase (decreases), lactate dehydrogenase (increases) and gamma-glutamyl transpeptidase (increases) associated with histopathological changes in testes, epididymides and seminal vesicles, were also observed. Accumulation of nickel in testes, epididymides and seminal vesicles was also observed. The study reveals that the oral exposure to nickel may affect the histology of testes, epididymides, seminal vesicles and sperms morphology. These testicular and spermatotoxic changes may be responsible for observed male mediated developmental toxic effects.

Alteration of testicular biochemistry during protein restriction in nickel treated rats.
Das K. K. and Dasgupta S.
Biol Trace Elem Res. 1997;60(3):243-9.

Nickel sulfate (2.0 mg/100 g.b.wt) dissolved in double-distilled water was administered (i.p.) on alternate days for ten doses to normal protein-fed and protein-restricted Wister strain albino rats (b.wt. 160 +/- 5 g). Two groups were used: one with normal protein diet, whereas the other with protein-restricted diet served as control. Twenty-four hours after the last treatment, the animals were sacrificed by decapitation. Tissues such as the testes, seminal vesicles, epididymis (Cauda and Caput) and prostate were dissected out, wiped clean, and stored at -20 degrees C until analysis. Lactate dehydrogenase (LDH) activities, glutamate oxaloacetate transaminase (GOT) activities, glycogen content, cholesterol content, and total protein content of the testes were estimated. Nickel sulfate administration significantly decreased the body weight of both normal protein-fed and protein-restricted groups of animals; the organ weights were also decreased. Significant decrease of LDH activity was observed, but GOT activity was not altered significantly. Testicular glycogen and cholesterol increased significantly in both experimental groups, but total protein content decreased. Nickel sulfate seems

to have an adverse effect on the male reproductive system in both groups of animals fed with normal protein (18% casein) diet and protein restricted (5% casein) diet.

Haemodynamic effect of nickel chloride in pregnant rats.
Szakmáry E, Morvai V, Náray M, Ungváry G.
Acta Physiol Hung. 1995;83(1):3-12.

Non-pregnant and pregnant CFY rats were given 3 mg/kg nickel chloride or physiological saline by gavage daily for eight days during days 7-14 of organogenesis. The haemodynamic investigations were carried out using 113Sn labelled microspheres. Nickel concentrations in maternal and fetal blood, as well as in amniotic fluid were determined by atomic absorption spectrophotometry. It was found, that nickel crossed the placenta, appeared in the fetal blood and amniotic fluid, where its concentration depended on the dose given to the pregnant animal and the nickel concentration of the maternal blood. Nickel chloride influenced neither the systemic haemodynamic parameters (arterial blood pressure, total peripheral resistance--TPR, cardiac index) nor the values of the organ (including the placenta) circulation indices, neither in the pregnant nor in the non-pregnant animals. It is concluded that in the pathomechanism of embryotoxicity (causing weight gain retardation) and teratogenicity (causing major anomalies of the uropoietic apparatus) of nickel, demonstrated earlier, the assumed effects of nickel on maternal and placental circulation probably do not play role (as such effects could not be detected). The direct embryo-damaging effect of nickel crossing the placenta (direct cytotoxic effect) may be held responsible for the embryotoxicity and teratogenicity of nickel.

Effects of chelating agents on testicular toxicity in mice caused by acute exposure to nickel.
Xie J., Funakoshi T., Shimada H. and Kojima S.
Toxicology. 1995;103(3):147-55.

N-Benzyl-D-glucaminedithiocarbamate (BGD), diethyldithiocarbamate (DDTC), dihydroxyethyldithiocarbamate (DHED), trans-1,2-cyclohexanediamine N,N,N',N'-tetraacetic acid (CDTA), and meso-2,3-dimercaptosuccinic acid (DMSA) were studied for their protective effects against the testicular toxicity in mice induced by acute exposure to nickel (Ni). Mice were injected intraperitoneally with NiCl2 (5 mgNi/kg) and 30 min or 24 h later, they were injected intraperitoneally with chelating agents (400 mumol/kg). Ni injection increased lipid peroxidation and concentrations of Ca and Fe in the testes, liver, and kidney, and decreased the testicular weight and the fertility rate. At

30 min after Ni treatment, the chelating agents other than CDTA effectively depressed Ni concentration in the testes. At 24 h after Ni treatment, DMSA, BGD, and DDTC were effective in mobilizing Ni from the testes. DMSA, BGD, and CDTA significantly prevented the increase in the lipid peroxidation, the increase in the concentrations of Ca and Fe in the testes, liver, and kidney, and the decrease in the fertility rate caused by Ni injection. Treatment with DMSA or BGD was more effective than that with the others in decreasing the testicular Ni concentration, resulting in effective protection against Ni-induced testicular damage.

Perinatal toxicity associated with nickel chloride exposure.
Smith M. K., George E. L., Stober J. A., Feng H. A. and Kimmel G. L.
Environ Res. 1993;61(2):200-11.

Several reports have suggested that soluble nickel salts may affect development. In this study female Long-Evans rats drank nickel chloride solutions (0, 10, 50, or 250 ppm Ni) for 11 weeks prior to mating and then during two successive gestation (G1, G2) and lactation (L1, L2) periods. Pups were observed until weaning; breeder males were unexposed. Dams drinking 250 ppm consumed less liquid and more food per kilogram body weight than did controls (liquid: prebreeding, G1, and G2; food: prebreeding, G2 and L2). Maternal weight gain was reduced during G1 in the high- and middle-dose groups; indices of reproductive performance were comparable across groups. Pup birth weight was unaltered by treatment and weight gain was reduced only in male pups exposed to 50 ppm Ni during L1. The frequency of perinatal death is the most significant toxicologic finding of the study. The proportion of dead pups per litter was significantly elevated at the high dose in L1 and at 10 and 250 ppm in L2 (50 ppm, P = 0.076), with a dose-related response in both experimental segments. The number of dead pups per litter was significantly increased at each dose in L2. Prolactin levels in pups were unchanged by treatment and were reduced in dams at the high dose. We conclude that 10 ppm Ni represents the lowest observed adverse effect level (LOAEL) in this study.

Effects of nickel chloride on lactating rats and their suckling pups, and the transfer of nickel through rat milk.
Dostal L. A., Hopfer S. M., Lin S. M. and Sunderman F. W., Jr.
Toxicol Appl Pharmacol. 1989;101(2):220-31.

The excretion of nickel into rat milk following subcutaneous (sc) doses of nickel chloride (NiCl2) and the effects on the lactating rat and her suckling pups were determined. Plasma and milk Ni concentrations increased in a dose-dependent manner 4 hr after single doses of 0, 10, 50, or 100 mumol NiCl2/kg to lactating rats, giving milk/plasma Ni ratios of 0.02. Peak plasma Ni concentrations were reached 4 hr after injection, while milk Ni increased until 12 hr and remained elevated at 24 hr. Dosing for 4 days at 50 or 100 mumol NiCl2/kg/day led to higher milk/plasma Ni ratios of 0.10. These doses of NiCl2 had no effect on body weight but caused decreased food consumption, thymic atrophy, and a small increase in hepatic lipid peroxidation in the dams. Significant alterations in milk composition, which were not due to decreased food consumption as determined in pair-fed rats, included increased milk solids (42%) and lipid (110%), and decreased milk protein (29%) and lactose (61%). NiCl2 treatment also caused significant decreases in mammary RNA content and the RNA/DNA ratio compared to both ad libitum-fed and pair-fed rats, indicating that milk synthetic activity was reduced by NiCl2. Pups suckling the NiCl2-treated dams had plasma Ni concentrations of 24 and 48 micrograms/liter in the 50 and 100 mumol/kg dose groups, respectively, and had decreased liver weight but no changes in hepatic lipid peroxidation or thymus weight. The results indicate that high doses of NiCl2 led to the excretion of Ni into rat milk and changes in milk quality and production. Reductions in liver weight in the suckling pups were also observed which may have been due to nickel exposure or to changes in milk composition.

Nickel- and cadmium-induced fetal myocardial changes in the mouse: the hazards of cigarette smoke in pregnancy.
Patai K. and Balogh I.
Acta Chir Hung. 1988;29(4):315-21.

The prolonged effect of nickel chloride and cadmium chloride on the rat fetal myocardium was studied experimentally administered to the pregnant mother through a gastric tube in doses of 12.5 mg/b. wt. It could be demonstrated that, due to nickel administration, changes simulating cardiomyopathy and severe mitochondrial lesions

developed and the number of collagenous fibres and glycogen granules accumulated, while as a result of cadmium chloride administration, changes were apparent mainly in the endothelial cells, but with simultaneous mitochondrial impairments, too. Nickel and cadmium are contained by cigarette smoke. Based on experimental studies, authors propose new arguments on the damaging effect of smoking of pregnant women.

The acute toxicity and teratogenicity of nickel in pregnant rats.
Mas A., Holt D. and Webb M.
Toxicology. 1985;35(1):47-57.

The increase susceptibility of the pregnant rat to intraperitoneally administered nickel (Ni) is apparent at 12 and 19 days of pregnancy and cannot be due, therefore, to the increase in total body weight. Teratogenic malformations occur when Ni is administered during organogenesis and are maximal at dose levels that are toxic for the dam. The yolk sac and chorioallantoic placentas accumulate Ni, but this does not prevent the transport of the metal to the embryo or foetus. The Ni concentrations in the conceptuses decrease more slowly with time than those in the maternal organs. In the foetuses, the decrease in concentration is due to the increase in weight, since the content of Ni increases between 4 h and 24 h. Foetal uptake of [14C]thymidine, [3H]leucine and 65Zn is unaffected at 3 h after the injection of the dam with 4 mg Ni/kg body wt. Incorporation of [3H]leucine into foetal protein, but not the incorporation of [14C]-thymidine into DNA, is decreased at this time. A major effect of treatment with this teratogenic dose is an increase in the maternal plasma glucose concentration which, in turn, alters the supply of the sugar to the foetus. The possible relevance of temporary foetal hyperglycaemia to teratogenesis is discussed.

Application of the in vitro embryo culture to the study of the mutagenic effects of nickel in male germ cells.
Jacquet P. and Mayence A.
Toxicol lett. 1982;11(1-2):193-7.

In vitro embryo cultures were utilized to determine the mechanism of preimplantation loss of embryos derived from matings 3 and 4 weeks after treatment of male Balb/c mice with 56 mg/kg nickel nitrate. Treated and control males were allowed to mate with superovulated females weekly for 5 weeks following treatment, and the number of cleaved eggs as well as development of embryos to blastocysts and implantation were determined. Controls included also males treated with a dose of 40 mg/kg nickel nitrate which previously had been shown to be ineffective in the dominant lethal test. Fertilizing

capacity of the spermatozoa as well as development of the cultured embryos were not influenced by a dose of 40 mg/kg nickel nitrate. A dose of 56 mg/kg significantly reduced, the fertilization rate 3 and 4 weeks after treatment but did not affect development of 2-cell embryos. These results demonstrate that the preimplantation loss induced by nickel treatment of males is due to toxic effect on spermatids and spermatogonia and not to a clastogenic action.

Nickel toxicity in early embryogenesis in mice.
Storeng R. and Jonsen J.
Toxicology. 1981;20(1):45-51.

The development of mouse embryos was studied after intraperitoneal injection of nickel chloride in the preimplantation period. A single intraperitoneal injection of NiCl2 . 6H2O in 0.154 M NaCl corresponding to 20 mg/kg body wt was given to groups of female mice on days 1, 2, 3, 4, 5 or 6 of gestation. Control groups were injected with 0.154 M NaCl. Caesarean section was performed on day 19 of gestation and the following parameters were recorded: implantation frequency, frequency of early and late resorptions, frequency of liver normal fetuses, abnormal fetuses and stillborns, and the weight of each fetus. The implantation frequency of females treated with nickel chloride on the first day of gestation was significantly lower than that of the controls. The size of the litters in the control groups was larger than that of the nickel treated dams, significant difference being observed on days 1, 3 and 5. NiCl2 . 6H2O injection also resulted in diminished body weights of fetuses on day 19 of gestation. The groups of nickel treated mice had a larger frequency of both early and late resorptions and the frequency of stillborn and abnormal fetuses exceeded that of the control groups. This study shows that, by the procedure used, nickel chloride may influence mouse embryos during the passage through the oviduct with subsequent effect on the development after implantation.U

Teratogenicity and embryotoxicity of nickel carbonyl in Syrian hamsters.
Sunderman F. W., Jr., Shen S. K., Reid M. C. and Allpass P. R.
Terato Carcin Mutagen. 1980;1(2):223-33.

Nickel carbonyl was administered to groups of pregnant hamsters by inhalation (0.06 mg Ni(CO)4/liter/15 min) on days 4, 5, 6, 7, or 8 of gestation. The dams were killed on day 15 of gestation, and the fetuses were examined for malformations. Exposure to Ni(CO)4 on days 4 or 5 of gestation resulted in malformations in 5.5% (8/146) and 5.8% (10/171) of the progeny, respectively (P less than 0.05, versus 0/95 in controls). The

proportions of litters with malformed fetuses were 33% (4/12) and 24% (4/17) in dams exposed to Ni(CO)4 on days 4 and 5 of gestation (P less than 0.05, versus 0/9 in litters of control dams). Progeny of dams exposed to Ni(CO)4 on days 4 and 5 included 9 fetuses with cystic lungs, 7 fetuses with exencephaly, 1 fetus with exencephaly plus fused rib, and 1 fetus with anophthalmia plus cleft palate. Hemorrhages into serous cavities were found in 18% (26/146) and 25% (42/171) of fetuses of dams exposed to Ni(CO)4 on days 4 or 5 of gestation. Such hemorrhages were not observed in controls. In progeny of dams exposed to Ni(CO)4 on days 6 or 7 of gestation, there was 1 fetus with fused ribs and there were 2 fetuses with hydronephrosis. In another experiment, pregnant hamsters were exposed to inhalation of Ni(CO)4 (0.06 mg/liter/15 min) on day 5 of gestation; these dams were permitted to deliver their litters and to nurse their pups. On the day of delivery, there was no significant difference in the average number of live pups in the Ni(CO)4-exposed litters compared to control litters. Neonatal mortality was increased in Ni(CO)4-exposed litters; by day 4 postpartum, the number of live pups averaged 7.6 +/- 1.5 in Ni(CO)4-exposed litters (P less than 0.01 versus 9.6 +/- 1.8 pups in control litters). This study demonstrates that Ni(CO)4 is teratogenic and embryotoxic in Syrian hamsters.

Teratogenic effects of nickel chloride on embryonic mice and its transfer to embryonic mice.
Lu C. C., Matsumoto N. and Iijima S.
Teratology. 1979;19(2):137-42.

Administration of nickel chloride to the pregnant mice on the seventh to eleventh day of their gestational period, resulted in significant embryotoxic effects in terms of an increased resorption rate, a decreased fetal weight, delay in skeletal ossification and high incidence of malformation. Among the cases of fetal malformation, the following malformations were observed to occur at a higher rate of incidence: acephalia, exencephaly, cerebral hernia, open eyelid, cleft palate, micromelia, ankylosis of the extremity, club foot and skeletal anomalies. Most skeletal anomalies were in the form of vertebral and/or rib fusions and were found mostly at thoracic and lumbar levels. The concentration of nickel retained in embryonic tissues was 800 times higher in the exposed compared to control groups and indicated that increased tissue levels of nickel chloride had a toxic influence on the developing embryo.

Eye malformations in rats: induction by prenatal exposure to nickel carbonyl.

Sunderman F. W., Jr., Allpass P. R., Mitchell J. M., Baselt R. C. and Albert D. M.
Science. 1979;203(4380):550-3.

Exposure of pregnant rats to inhalation of nickel carbonyl on days 7 or 8 of gestation frequently causes the progeny to develop ocular anomalies, including anophthalmia and microphthalmia. The incidence of extraocular anomalies is very low. The specificity of nickel carbonyl for induction of ocular anomalies in rats appears to be unique among known teratogenic agents.

Effect of nickel sulphate on male rats.
Mathur A. K., Datta K. K., Tandon S. K. and Dikshith T. S.
Bull Environ Contamin Toxicol. 1977;17(2):241-8.

Cytopathological and histopathological changes in skin, liver, kidney and testis of rats due to nickel sulphate have been studied experimentally. Skin of nickel sulphate painted rats showed hyperkeratinization, vacuolization, hydropic degeneration of basal layer and atrophy of epidermis. The testis showed degeneration and oedema of seminiferous tubules, while liver showed areas of focal necrosis, congestion and dilatation of sinusoids.

Biochemical and morphological changes in some organs of rats in nickel intoxication.
Mathur A. K., Chandra S. V., Behari J. and Tandon S. K.
Arch Toxicol. 1977;37(2):159-64.

Biochemical and histological alterations in liver, kidney, testis and myocardium of albino rats were investigated at various intervals after daily administration of nickel sulphate. No significant changes were observed during the initial periods of the treatment, but longer exposures produced marked enzymatic and histopathological alterations in all the four organs examined. The severity of the histological lesions was found to be directly related to the duration of the treatment.

B. Studies reporting no developmental or reproductive toxicity

In vitro embryotoxicity testing of metals for dental use by differentiation of embryonic stem cell test.
Imai K. and Nakamura M.
Cong Anomal. 2006;46(1):34-8.

We examined embryotoxicity using the embryonic stem cell test (EST) protocol. Tests were conducted using standard reagents for the atomic absorption measurement of 11 metal ions, silver, cobalt, chromium, copper, mercury, nickel, palladium, antimony, tin, vanadium, and zinc from among metals comprising dental alloys. In addition, for four metals like silver, cobalt, chromium, and nickel, the tests were also conducted using a test solution extracted from powder in the cell culture medium. The embryotoxic potential was obtained from a biostatistics-based prediction model, which was calculated from three endpoints, the ID50, IC50ES and IC(50)3T3. Data with the standard reagents showed that chromium and mercury ions corresponded to class 3, that is, having a strong embryotoxicity, while antimony, tin, and vanadium ions exhibited a weak embryotoxicity. The other metal ions demonstrated no embryotoxicity. On the other hand, when extracts of metal powder in cell culture solutions were used, silver exhibited a weak embryotoxicity while all other metals exhibited no embryotoxicity. In the future, it will be important to clarify the embryotoxicity of the many dental materials that are in use today. In addition, it is necessary to develop substances to ensure they have no toxicity before use in dental applications.

Combined effects of cadmium and nickel on testicular xenobiotic metabolizing enzymes in rats.
Işcan M, Ada AO, Coban T, Kapucuoğlu N, Aydin A, Isimer A
Biol Trace Elem Res. 2002;89(2):177-90.

When male rats were given a single dose of cadmium (Cd) (3.58 mg CdCl2 x H2O/kg, i.p.) 72 hr prior to sacrifice, the testicular 7-ethoxyresorufin O-deethylase (EROD) and glutathione S-transferase (GST) activities toward the substrates 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), ethacrynic acid (EAA), 1,2-epoxy-3-(p-nitrophenoxy)-propane (EPNP), and cumene hydroperoxide (CHPx) decreased significantly as compared to controls. Cd also inhibited reduced glutathione (GSH) level while increasing the lipid peroxidation (LP) level significantly. When the animals were given a single dose of nickel (Ni) (59.5 mg NiCl2 x 6H2O/kg, i.p.) 16 hr

prior to sacrifice, significant decreases were observed in EROD and GST activities toward CDNB, EAA, EPNP, and CHPx, and GSH level. No significant alterations were noted in DCNB GST activity and LP level by Ni. For the combined treatment, rats received the single dose of Ni 56 hr after the single dose of Cd and were killed 16 hr later. In these animals, lesser depressions were observed on EROD activity and LP level than those of Cd alone. The combination of metals significantly inhibited GST activities and GSH level but not to a greater degree than noted by Cd or Ni alone. Plasma testosterone levels of Cd-, Ni-, and combination-treated rats decreased significantly compared to controls. The strongest depression was achieved by Cd alone. Cd, both alone and in combination with Ni, increased the tissue Ni uptake significantly. Ni, however, did not produce such an effect on the tissue uptake of Cd in either case. Cd treatment caused interstitial edema and coagulation necrosis in seminiferous tubules and also caused fibrinoidal necrosis in vascular endothelium. Ni treatment did not produce any pathological testicular alterations compared to controls. Combined treatment produced fewer pathological alterations (i.e., only interstitial edema) than that of Cd treatment. These results reveal that the combination of Cd and Ni does not have a synergistic effect on testicular xenobiotic metabolizing enzymes, and in contrast, Ni has an ameliorating effect on pathological disturbances caused by Cd alone in the rat testis.

Toxicity and bioaccumulation of nickel sulfate in Sprague-Dawley rats following 13 weeks of subchronic exposure.
Obone E., Chakrabarti S. K., Bai C., Malick M. A., Lamontagne L. and Subramanian K. S.
J Toxicol Environ Health Part A. 1999;57(6):379-401.

Adult male Sprague-Dawley rats were given 0, 0.02, 0.05, and 0.1% nickel sulfate (NiSO4-6H2O) or 0, 44.7, 111.75, and 223.5 mg Ni/L, respectively, in their drinking water for 13 wk. Twenty-four hours following the end of such treatment, all animals survived and no apparent clinical signs of toxicity were noted. The final mean body weights of various nickel sulfate-treated rats were not significantly decreased except for the 0.1% nickel sulfate treated group when compared to those in the control. The absolute and relative organ weights were either increased or decreased or remained unchanged, depending on the organ and the dose of nickel sulfate. Total plasma proteins, plasma albumin and globulins, and plasma glutamic pyruvic transaminase activity were all significantly decreased in 0.1% nickel sulfate-treated rats. Lymphocyte subpopulations (T and B cells) were induced at lower dose levels, but suppressed at the highest (0.1%) dose group. A significant decrease in urine volume and an increase in BUN were observed at the highest dose group. Biochemical analysis of bronchoalveolar

lavage fluid and lung tissue showed some lung damage, whereas no damage to the testis or DNA in liver and kidneys were found. No gross or microscopic changes were seen in any of the various tissues examined. The relative order of bioaccumulation of nickel in different organs of rats when treated at 0.1% nickel sulfate (223.5 mg Ni/L) was kidneys > testes > lung = brain > spleen > heart = liver. But with regard to order of toxicity, both immune and pulmonary systems were found to be very sensitive targets, followed by kidney.

Toxic effect of heavy metals on cells isolated from the rat adrenal and testis.
Ng T. B. and Liu W. K.
In Vitro Cell Dev Bio. 1990;26(1):24-8.

Heavy metals including mercury, cadmium, cobalt, and copper (100 microM) exerted an adverse effect on the viability of isolated rat adrenal capsular (zona glomerulosa), adrenal decapsular (fasciculata and reticularis), and Leydig cells of the testis, with mercury being the most potent. Due to the decreased cell viability there was a parallel reduction in corticotropin-stimulated corticosterone production by adrenal decapsular cells and luteinizing hormone-stimulated testosterone production by Leydig cells. The results indicated a direct toxic action of these heavy metals on steroid-producing cells in the adrenal gland and the testis. Other metals tested, including lead, zinc, aluminum, chromium, iron, nickel, and lithium, did not exert any deleterious effect on cell viability or hormone-induced steroidogenesis in adrenal and Leydig cells when tested up to a concentration of 100 microM.

Negative test for transplacental carcinogenicity of nickel subsulfide in Fischer rats.
Sunderman F. W., Jr., McCully K. S. and Rinehimer L. A.
Res Commun Chem Pathol Pharmacol. 1981;31(3):545-54.

Transplacental carcinogenicity of nickel subsulfide (Ni3S2) was tested by im administration of Ni3S2 (20 mg) to 8 pregnant Fischer rats on day 6 of gestation. Control dams received a similar injection of the vehicle. The progeny at risk for tumor development comprised 50 offspring (17 males, 33 females) of Ni3S2-treated dams and 53 offspring (29 males, 24 females) of control dams. Body weights of progeny of Ni3S2 treated dams were lower than progeny of control dams, but sex-specific cumulative mortality rates in the two groups did not differ significantly. By age 26 months, 2 malignant and 2 benign tumors developed in progeny of Ni3S2-treated dams, versus 3

malignant and 2 benign tumors in progeny of control dams. Hence, administration of Ni3S2 to pregnant rats had no significant effect upon tumor incidence in the progeny.

C. Related articles

Municipal landfill leachate-induced testicular oxidative damage is associated with biometal accumulation and endocrine disruption in rats.
Adedara I. A., Awogbindin I. O., Adesina A. A., Oyebiyi O. O., Lawal T. A. and Farombi E. O.
Arch Environ Contamin Toxicol. 2015;68(1):74-82.

Improper management of hazardous wastes adversely impacts the environment and the public health. The present study was aimed at investigating the influence of Olushosun municipal landfill leachate (OMLL) from Ojota in the Lagos State of Nigeria on testicular function by assessing the plasma concentrations of reproductive hormones, testicular biometal levels, and antioxidant levels as well as observing the histological alterations in testes and epididymides of rats after exposure to 0, 12.5, and 25% OMLL in drinking water for 7 days. Exposure to OMLL significantly decreased the daily fluid intake, but it resulted in testicular biometal accumulation as follows: lead > cadmium > nickel > iron > copper. Acute exposure to OMLL induced oxidative stress and increased the activities of marker enzymes of testicular function but markedly decreased the circulatory concentrations of luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, thyroid-stimulating hormone, triiodothyronine, and thyroxine. Testicular and epididymal degeneration with significant decrease in sperm quality and quantity were observed in OMLL-exposed rats. Collectively, the data presented herein indicate that exposure to OMLL-induced testicular dysfunction associated with biometal accumulation and endocrine disruption in rats. If the effects can be extrapolated to humans, OMLL may present significant health implications for individuals exposed to OMLL-contaminated substances.

Reproductive impacts and physiological adaptations of zebrafish to elevated dietary nickel.
Alsop D., Lall S. P. and Wood C. M.
Comp Biochem Physiol Toxicol Pharmacol. 2014;165:67-75.

Nickel (Ni) concentrations in the environment can rise due to human industrial activities. The toxicity of waterborne Ni to aquatic animals has been examined in a number of

previous studies; however, little is known about the impacts of elevated dietary Ni. In the present study, zebrafish were chronically fed diets containing two concentrations of Ni [3.7 (control) and 116 µg Ni/g diet]. Ni-exposed males, but not females, were significantly smaller (26%) compared to controls at 80 days. In addition, total egg production was decreased by 65% in the Ni treatment at 75-78 days of the experiment. Ni was ubiquitously distributed in control animals (similar to previous studies), and concentrations varied between tissues by 15-fold. Ni exposure resulted in modest but significant Ni accumulation in some tissues (increases were highest in brain, vertebrae and gut; 44%, 34% and 25%, respectively), an effect observed only at 80 days. The limited Ni accumulation may be due to (1) the lack of an acidified stomach in zebrafish and/or (2) the efficient upregulation of Ni transport and excretion mechanisms, as indicated by the 4.5-fold increase in waterborne (63)Ni uptake by Ni-exposed fish. Eggs from Ni-exposed adults had Ni concentrations that were 5.2-fold higher than controls. However, by 4 days post fertilization, larvae had similar Ni concentrations as controls, demonstrating a capacity for rapid Ni depuration. Larvae from Ni-exposed adults were also more resistant to waterborne Ni (35% increase in the 96-h LC50 over controls). In conclusion, elevated dietary Ni significantly affected zebrafish reproduction despite only modest tissue Ni accumulation. There were also indications of adaptation, including increased Ni uptake rates and increased Ni tolerance of offspring from Ni-exposed adults. Ni concentrations were particularly elevated in the brain with exposure; possible relations to growth and reproductive impacts require further study.

Changes of chemical chronic toxicity to Daphnia magna under different food regimes.
Pavlaki M. D., Ferreira A. L., Soares A. M. and Loureiro S.
Ecotoxicol Environ Safety. 2014;109:48-55.

In aquatic ecosystems several stressors may act together and affect the life traits of organisms. Pesticide runoffs are usually associated with high inputs of organic matter and depletion of oxygen in aquatic systems. This study aimed at combining anthropogenic stress (chemicals) and natural stress (food availability) and evaluates their joint effect to the life traits of Daphnia magna. The neonicotinoid insecticide imidacloprid and the heavy metal nickel chloride were used and a 21 d chronic test was carried out to obtain reproduction and growth data. The conceptual model Independent action, usually used for assessing response patterns in chemical mixtures, was used for data interpretation. Results showed an increase in the reproduction and growth pattern of D. magna as food levels increased. Both chemicals significantly impaired the reproduction as well as the somatic growth of the organism while the same happened

with food concentrations lower than 3x10(5) cells/mL. It was also observed that food availability did not change the toxicity of imidacloprid and nickel chloride when food levels were higher than 3x10(5) cells/mL. When combined with low food levels, imidacloprid showed a slight increase in toxicity, showing that daphnids become more sensitive with reduced food availability, however in a non-significant way. However, toxicity of nickel appeared to be independent of the food level. Both chemicals induced mortality to the organisms exposed in the absence of food only at the end of the test.

Does mechanism matter? Unrelated neurotoxicants converge on cell cycle and apoptosis during neurodifferentiation.
Slotkin T. A. and Seidler F. J.
Neurotoxicol Teratol. 2012;34(4):395-402.

Mechanistically unrelated developmental neurotoxicants often produce neural cell loss culminating in similar functional and behavioral outcomes. We compared an organophosphate pesticide (diazinon), an organochlorine pesticide (dieldrin) and a metal (Ni(2+)) for effects on the genes regulating cell cycle and apoptosis in differentiating PC12 cells, an in vitro model of neuronal development. Each agent was introduced at 30µM for 24 or 72h, treatments devoid of cytotoxicity. Using microarrays, we examined the mRNAs encoding nearly 400 genes involved in each of the biological processes. All three agents targeted both the cell cycle and apoptosis pathways, evidenced by significant transcriptional changes in 40-45% of the cell cycle-related genes and 30-40% of the apoptosis-related genes. There was also a high degree of overlap as to which specific genes were affected by the diverse agents, with 80 cell cycle genes and 56 apoptosis genes common to all three. Concordance analysis, which assesses stringent matching of the direction, magnitude and timing of the transcriptional changes, showed highly significant correlations for pairwise comparisons of all the agents, for both cell cycle and apoptosis. Our results show that otherwise disparate developmental neurotoxicants converge on common cellular pathways governing the acquisition and programmed death of neural cells, providing a specific link to cell deficits. Our studies suggest that identifying the initial mechanism of action of a developmental neurotoxicant may be strategically less important than focusing on the pathways that converge on common final outcomes such as cell loss.

In vitro effect of nickel on bovine spermatozoa motility and annexin V-labeled membrane changes.

Lukac N., Bardos L., Stawarz R., Roychoudhury S., Makarevich A. V., Chrenek P., Danko J. and Massanyi P.
J Appl Toxicol. 2011;31(2):144-9.

In this study the effect of in vitro culture of bovine spermatozoa with nickel (NiCl(2)) on spermatozoa motility and membrane changes was analyzed. The spermatozoa motility significantly decreased after 120 min of culture at the concentration of 1000 µM Ni ml(-1) ($P < 0.05$) and after 240 min of culture at the concentration of 500 and 1000 µM Ni ml(-1) ($P < 0.001$) as compared with control. The progressive motility was the highest in the control group and in the groups with the lowest nickel concentrations (7.8 and 125 µM Ni ml(-1)). The progressive spermatozoa motility was significantly altered even after 30 min of culture in the group with the highest nickel concentration (1000 µM Ni ml(-1)). A significant decrease in progressive motility from the concentration of 250 µM Ni ml(-1) was detected after 240 min of culture. Concentrations from 125 µM Ni ml(-1) in various time periods of culture stimulated spermatozoa motility after 30 min ($P < 0.001$), but later an inhibitory effect was noted. After 240 min of in vitro spermatozoa culture with 125 µM Ni ml(-1) a typical Annexin V fluorescence reaction was detected. Fluorescence was detected in mitochondrial segment of bovine spermatozoa. In spermatozoa exposed to higher nickel concentrations the Annexin V-positive reaction was detected also on the spermatozoa head membrane. In the group with the highest concentration and the longest time of exposure (1000 µM Ni ml(-1); 240 min) the apoptotic Annexin-positive regions were detected not only in the mitochondrial part, but also in the spermatozoa head (acrosomal and postacrosomal part), showing significant alteration of spermatozoa membrane integrity.

Nickel toxicity in embryos and larvae of the South American toad: effects on cell differentiation, morphogenesis, and oxygen consumption.
Sztrum A. A., D'Eramo J. L. and Herkovits J.
Environ Toxicol Chem. 2011;30(5):1146-52.

Nickel, a widely distributed heavy metal in the biosphere, produces systemic, carcinogenic, and teratogenic effects. The objectives of the present study are to report the acute, short-term chronic, and chronic toxicity of Ni in Rhinella arenarum embryos as well as the stage-dependent susceptibility to this heavy metal, including oxygen consumption, teratogenesis, and adverse effects on cell differentiation processes. The

stages evaluated were blastula (S.7), gastrula (S.11), tail bud (S.17), fin circulation (S.22), and complete operculum (S.25), in this last case by means of toxicity profile curves. Nickel increases its adverse effects gradually, with a maximum value after 96 h. The 50% lethal concentrations (LC50s) for 96, 168, and 240 h at S.25 were 1.14, 0.60, and 0.48 mg Ni^{2};(+) /L, respectively; S.11 and S.22 were the least and most susceptible to Ni with, LC50s 96 h of 6.12 and 0.19 mg Ni^{2};(+) /L, respectively. A reduction of approximately 25% in oxygen consumption anticipates lethal effects from S.17 onward. The main teratogenic effects were retarded growth and development, extremely severe axis incurvations, persistent yolk plug, asymmetry, microcephaly and mouth and gill agenesia, and limited neuromuscular activity. Ciliated cells were not functional. The possibility of associating the remarkable stage-dependent susceptibility to Ni with environmental changes during the evolutionary process is also considered.

Nickel induced structural and functional alterations in mouse Leydig cells in vitro.
Kročková J. Z., Massányi P., Sirotkin A. V., Pivko J., Makarevich A. V., Lukáč , Capcarová M., Toman R. and Poláková Z.
J Trace Elemen Med Bio. 2011;25(1):14-8.

The present study was aimed at investigating effects of nickel (NiCl(2)) on secretion of testosterone (T), cell viability, ultrastructure and apoptosis in mouse Leydig cells. Testosterone release was measured after 48h of culture with 15.67, 31.25, 62.5, 125, 250, 500 and 1000μmol/L NiCl(2) or without NiCl(2) using radioimmunoassay. Cell viability was assessed by a MTT (metabolic activity assay). Quantification of apoptotic cells was performed using TUNEL assay and the ultrastructural changes were analyzed using transmission electron microscopy. The viability was decreased after addition of ≥250μmol/L NiCl(2). A concentration-dependent depression of T production was observed. The percentage of apoptotic cells was significantly increased only after addition of 125, 250 and 1000μmol/L NiCl(2). After addition of ≥250μmol/L NiCl(2) higher incidence of euchromatin was observed. Lipid droplets and vacuoles in cytoplasm were increased after addition of ≥125μmol/L NiCl(2). NiCl(2) induced decrease in numbers of mitochondria and smooth endoplasmic reticulum after treatment with ≥500μmol/L NiCl(2). Our findings suggest a negative effect of NiCl(2) on steroidogenesis, viability, apoptosis and ultrastructure of mouse Leydig cells.

The characteristics of placental transfer and tissue concentrations of nickel in late gestational rats and fetuses.

Hou Y. P., Gu J. Y., Shao Y. F., Song Y. F., Jing Y. H., Wu W. S. and Pu S.
Placenta. 2011;32(3):277-82.

The dynamics of nickel (Ni) uptake, transfer, retention and clearance in fetuses and late gestational rats were investigated by assessing its distributions in placenta, maternal and fetal organs and tissues during the 24 h period after a single dose of (63)Ni intraperitoneal injection on gestational day 20. Peak (63)Ni radioactivity was detected at 0.5 h in maternal blood, at 3 h in placenta, fetal membranes, fetal blood, fetal heart, maternal kidney, lung, stomach, liver and brain, at 9 h in fetal kidney, stomach, liver and brain, and lastly at 24 h in fetal lung and amniotic fluid. The maximal (63)Ni radioactivity among all samples was detected consistently in the fetal membranes and placenta. The (63)Ni radioactivity in fetal blood was higher than that in maternal blood from 3 to 24 h. The fetal liver, heart, stomach and brain exhibited higher (63)Ni radioactivity than the corresponding maternal organs from 6 to 24 h. However, maternal kidney consistently exhibited significantly higher (63)Ni radioactivity than the fetal kidney. The (63)Ni in fetal lung and amniotic fluid increased throughout the period of experimental observation. These observations corroborated previous finding that nickel is actively transferred across the blood-placenta-barrier into fetus, but hardly from fetus to mother. Moreover, these results suggest that the placenta has a high affinity for nickel and its barrier does not protect the fetus from nickel exposure. The fact that nickel concentrations are higher in most fetal organs and tissues than in corresponding maternal organs and tissues in late gestation indicates that, unlike the dam, fetuses lack effective means for getting rid of excessive nickel due to its confined environment and relatively weak kidney functions. The situation is exacerbated by mother-to-fetus unidirectional transfer. Consequently, the fetuses are particularly vulnerable to the damaging effects of nickel.

Gestational age and dose influence on placental transfer of 63Ni in rats.
Wang X. W., Gu J. Y., Li Z., Song Y. F., Wu W. S. and Hou Y. P.
Placenta. 2010;31(4):305-11.

The effects of gestational age and dose of nickel exposure on regulating and influencing placental transfer were investigated. Pregnant rats on gestational day (GD) 12, 15 or 20 were injected intraperitoneally with saline, 64,320 or 640 kBq/kg body weight of (63)Ni. Twenty-four hours after administration, samples were harvested from each for measurement of radioactivity by liquid scintillation counting and for autoradiography. In

placenta, amniotic fluid and fetal membrane, (63)Ni concentrations increased with increasing doses and gestational age. In fetus, (63)Ni concentrations reached a maximum on GD 15 and then declined on GD 20 although they maintained a dose-dependency for each GD group. In fetal blood on GD 20, (63)Ni concentration increased dose-dependently and was higher than in maternal blood. The autoradiographs demonstrated that (63)Ni radioactivity was located within placental basal lamina, fetal bones and most organs. These findings suggest that the nickel uptake, retention and transport in placenta increase dose- and gestation age-dependently, and nickel transfer through placental barrier is primarily from mother into the fetus, but hardly from fetus to mother.

Disparate developmental neurotoxicants converge on the cyclic AMP signaling cascade, revealed by transcriptional profiles in vitro and in vivo.
Adigun A. A., Seidler F. J. and Slotkin T. A.
Brain Res. 2010;1316:1-16.

Cell-signaling cascades are convergent targets for developmental neurotoxicity of otherwise unrelated agents. We compared organophosphates (chlorpyrifos, diazinon), an organochlorine (dieldrin) and a metal (Ni(2+)) for their effects on neuronotypic PC12 cells, assessing gene transcription involved in the cyclic AMP pathway. Each agent was introduced during neurodifferentiation at a concentration of 30 microM for 24 or 72 h and we assessed 69 genes encoding adenylyl cyclase isoforms and regulators, G-protein alpha-and beta,gamma-subunits, protein kinase A subtypes and the phosphodiesterase family. We found strong concordance among the four agents across all the gene families, with the strongest relationships for the G-proteins, followed by adenylyl cyclase, and lesser concordance for protein kinase A and phosphodiesterase. Superimposed on this pattern, chlorpyrifos and diazinon were surprisingly the least alike, whereas there was strong concordance of dieldrin and Ni(2+) with each other and with each individual organophosphate. Further, the effects of chlorpyrifos differed substantially depending on whether cells were undifferentiated or differentiating. To resolve the disparities between chlorpyrifos and diazinon, we performed analyses in rat brain regions after in vivo neonatal exposures; unlike the in vitro results, there was strong concordance. Our results show that unrelated developmental neurotoxicants can nevertheless produce similar outcomes by targeting cell signaling pathways involved in neurodifferentiation during a critical developmental period of vulnerability. Nevertheless, a full evaluation of concordance between different toxicants requires evaluations of in vitro systems that detect direct effects, as well as in vivo systems that allow for more complex interactions that converge on the same pathway.

Oxidative and excitatory mechanisms of developmental neurotoxicity: transcriptional profiles for chlorpyrifos, diazinon, dieldrin, and divalent nickel in PC12 cells.
Slotkin T. A. and Seidler F. J.
Environ Health Perspect. 2009;117(4):587-96.

BACKGROUND: Oxidative stress and excitotoxicity underlie the developmental neurotoxicity of numerous chemicals.
OBJECTIVES: We compared the effects of organophosphates (chlorpyrifos and diazinon), an organo-chlorine (dieldrin), and a metal [divalent nickel (Ni2+)] to determine how these mechanisms contribute to similar or dissimilar neurotoxic outcomes.
METHODS: We used PC12 cells as a model of developing neurons and evaluated transcriptional profiles for genes for oxidative stress responses and glutamate receptors.
RESULTS: Chlorpyrifos had a greater effect on oxidative-stress-related genes in differentiating cells compared with the undifferentiated state. Chlorpyrifos and diazinon showed significant concordance in their effects on glutathione-related genes, but they were negatively correlated for effects on catalase and superoxide dismutase isoforms and had no concordance for effects on ionotropic glutamate receptors. Surprisingly, the correlations were stronger between diazinon and dieldrin than between the two organophosphates. The effects of Ni2+ were the least similar for genes related to oxidative stress but had significant concordance with dieldrin for effects on glutamate receptors.
CONCLUSIONS: Our results point to underlying mechanisms by which different organophosphates produce disparate neurotoxic outcomes despite their shared property as cholinesterase inhibitors. Further, apparently unrelated neurotoxicants may produce similar outcomes because of convergence on oxidative stress and excitotoxicity. The combined use of cell cultures and microarrays points to specific end points that can distinguish similarities and disparities in the effects of diverse developmental neurotoxicants.

The effects of dietary nickel exposure on growth and reproduction of Daphnia magna.
Evens R., De S. K. A. and Janssen C. R.
Aquatic Toxicol. 2009;94(2):138-44.

Although there is growing evidence that dietborne metals can be toxic to various aquatic species, there is still insufficient knowledge to integrate this information in environmental

risk assessment procedures. In this study, we investigated the effects of a 21-day exposure of Daphnia magna to a control diet (i.e. the green alga Pseudokirchneriella subcapitata containing < 4.0microgNi/g dry wt) and five diets with elevated Ni concentrations (i.e. the same alga contaminated with Ni burdens between 33.7 and 837microgNi/g dry wt). A significant accumulation of dietborne Ni in D. magna, i.e. between 49.6 and 72.5microgNi/g dry wt, was observed when they were fed with diets containing between 85.6 and 837microgNi/g dry wt. This was paralleled by a significant reduction of reproduction (by 33.1%), measured as the total number of juvenile offspring per female and growth (by 9.1%), measured as the carapax length of 21-day-old females. Life-history analysis showed that the time to first brood of Ni exposed organisms was between 7.8 and 8.2 days, and occurred 0.7-1.1 days earlier than for the control organisms (time to first brood=8.9 days). The number of offspring in the first brood was significantly reduced (by 21-33% compared to the control) in all dietary treatments. Longer exposure (> or =8.9 days, i.e. from the second brood onwards) led to a reduction of brood size only when given diets containing 85.6 and 837microgNi/g dry wt. The results suggest that a variety of mechanisms may be involved in the effects of dietary Ni exposure, including altered resource allocation or targeted reproductive inhibition. While Ni exposure clearly altered the quality of the diet (measured as essential omega3 polyunsaturated fatty acid content and C:P ratio), we found no conclusive evidence that these diet quality shifts could have affected growth or total reproductive output. More research is required to fully understand the mechanisms of Ni toxicity associated with the dietary exposure route.

Protein kinase C is a target for diverse developmental neurotoxicants: transcriptional responses to chlorpyrifos, diazinon, dieldrin and divalent nickel in PC12 cells.
Slotkin T. A. and Seidler F. J.
Brain Res. 2009;1263:23-32.

Unrelated developmental neurotoxicants can elicit similar functional outcomes, whereas agents in the same class may differ. We compared two organophosphate insecticides (chlorpyrifos, diazinon) with an organochlorine (dieldrin) and a metal (Ni(2+)) for similarities and differences in their effects on gene expression encoding subtypes of protein kinase C and their modulators, a cell signaling cascade that integrates the actions of neurotrophic factors involved in brain development. We conducted evaluations in PC12 cells, a model for neuronal development, with each agent introduced at 30 microM for 24 or 72 h, treatments devoid of cytotoxicity. Chlorpyrifos evoked by far the largest effect, with widespread upregulation of multiple genes; the

effects were greater during neurodifferentiation than when cells were exposed prior to differentiation. Diazinon had smaller and less widespread effects, consistent with its lesser long-term impact on synaptic function and behavior noted for in vivo exposures in developing rats. Surprisingly, the effects of diazinon, dieldrin and Ni(2+) showed basic similarities despite the fact that all three come from different classes of toxicants. Our findings provide some of the first evidence for a specific mechanistic cascade contributing to the cholinesterase-independent developmental neurotoxicant actions of chlorpyrifos and its differences from diazinon, while at the same time identifying mechanistic convergence between otherwise unrelated toxicants that provides predictions about common neurodevelopmental outcomes. These results further show how combined use of cell cultures and microarray technology can guide future in vivo work on diverse developmental neurotoxicants.

Nickel tissue residue as a biomarker of sub-toxic exposure and susceptibility in amphibian embryos.
Pérez-Coll, C. S. Sztrum, A. A. and Herkovits, J.
Chemosphere 2008: 74(1): 78-83.

Although low level exposure to physicochemical agents is the most common environmental scenario, their effects on living organisms are very controversial. However, there is an increasing need to integrate low level exposures from risk assessment to remediation purposes. This study focus on the possibility to employ Ni tissue residue values as biomarkers of sub-toxic exposure and susceptibility to this metal in a range of almost pristine to sub-toxic concentrations for Rhinella arenarum embryos. For that purpose, three batches of amphibian embryos were pretreated during 10 days with three increasing concentrations of Ni starting in 2, 8 and 20 microg Ni(2+) L(-1) and ending in 16, 64 and 160 microg Ni(2+) L(-1) (in natural fresh waters this value ranges from 2 to 10 microgL(-1); the LC(50)-24h for R. arenarum is 26.2mg Ni(2+) L(-1)). For the experimental conditions, the Ni tissue residue values at 360 h post exposure were 0.5, 2.1 and 3.6 microg Ni g(-1) embryo w/w, respectively, corresponding to BCFs of 31, 33 and 23. The susceptibility to Ni in those experimental embryos was evaluated by means of challenge exposures to three lethal concentrations of this metal (10, 20 and 30 mg Ni(2+) L(-1)), registering survival during the following 10 days of treatment. As a general pattern, the lower, intermediate and higher pretreatments with Ni resulted in enhanced, neutral and adverse effects on embryonic survival, respectively. Thus, sub-toxic exposure to Ni could modify the resistance of the amphibian embryo to this metal and Ni tissue residue values could be considered as biomarkers of both, exposure and susceptibility.

Comparative developmental toxicity of nickel to Gastrophryne carolinensis, Bufo terrestris, and Xenopus laevis.
Fort, D. J. Rogers, R. L. Thomas, J. H. Hopkins, W. A. and Schlekat, C.
Arch Environ Contam Toxicol. 2006: **51**(4): 703-710.

The early embryo-larval developmental toxicity of nickel (Ni) to 3 amphibian species, Xenopus laevis (South African clawed frog), Bufo terrestris (southern toad), and Gastrophryne carolinensis (eastern narrow-mouthed toad), was evaluated using a modified FETAX model. Studies were initiated from late blastulae stage (Nieuwkoop and Faber [NF] stage 10 or Gosner stage 12) and completed at a common embryological-based test termination point, which represented the completion of the major stages of organogenesis (NF stage 46 for Xenopus or Gosner stage 26 for the toads). Results indicated that, in terms of lethality, G. carolinensis was the most sensitive and X. laevis was the least sensitive of the species tested. The 4-d LC50 in X. laevis value was approximately 7.2- and 2.8-fold greater than the G. carolinensis and B. terrestris, respectively. In terms of malformation, X. laevis was the most sensitive and B. terrestris was the least sensitive of the species tested. The 7-d EC50 (malformation) in B. terrestris was 10.6- and 7.0-fold greater than X. laevis or G. carolinensis, respectively. The chronic value (ChV) for growth in X. laevis was nearly 4.5-fold less than the ChV for growth determined for B. terrestris. As with the malformation endpoint, X. laevis was more sensitive than the other species, which were nearly equisensitive. Overall, the present study provides new data regarding the toxicity of Ni to larval amphibian species, which may be useful in the establishment of new aquatic life criteria for Ni.

[Genetic effects induced by nickel sulfate in germline and somatic cells of WR mice].
Domshlak M. G., Elakov A. L. and Osipov A. N.
Genetika. 2005;41(7):894-901.

We examined the effects of nickel sulfate at doses 0.5 to 5.0 mg/kg (LD50) on the frequency of dominant lethal mutations and two-strand DNA breaks (TSBs) in germline cells and on an increase in frequency in gene mutations W(y) in pigment cells of first-generation mice. The results indicated that spermatogenesis stages most sensitive to nickel sulfate (at a dose of 1.0 mg/kg) are spermatozoids, early spermatids, late spermatocytes, and stem spermatogonia. No statistically significant increase in the total TSB level was detected in spermatozoids 4 weeks after exposure. At the same time, a

significant (P < 0.05) increase in percentage of cells with an extremely high level of DNA fragmentation (supposedly apoptotic cells) was observed upon exposure at a dose of 0.5 mg/kg. Nickel sulfate at doses of 5.0 and 1.0 mg/kg induced a marked increase in the c-kit gene expression in pigment cells of heterozygous first-generation WR mice as compared to control (P < 0.001). It was shown that the nonobservable adverse effect level (NOAEL) of nickel sulfate on the dominant lethal mutation frequency and gene mutations was 1/200 LD50, while the lowest observable adverse effect level (LOAEL) was 1/100 LD50.

Effects of chronic waterborne nickel exposure on two successive generations of Daphnia magna.
Pane E. F., McGeer J. C. and Wood C. M.
Environ Toxicol Chem. 2004;23(4):1051-6.

In a 21-d chronic toxicity test in which an F0 generation of Daphnia magna were exposed to waterborne Ni, the no-observable-effect concentration (for survival, reproduction, and growth) was 42 microg Ni L(-1), or 58% of the measured 21-d median lethal concentration (LC50) of 71.9 microg Ni L(-1) (95% confidence interval, 56.5-95.0). Chronic exposure to 85 microg Ni L(-1) caused marked decreases in survival, reproduction, and growth in F0 animals. In the F1 generation (daphnids born of mothers from the chronically exposed F0 generation), animals chronically exposed to 42 microg Ni L(-1) for 11 d weighed significantly less (20%) than controls, indicating increased sensitivity of F1 animals. Additionally, in this successive generation, significant decreases in whole-body levels of metabolites occurred following exposure to both 42 microg Ni L(-1) (decreased glycogen and adenosine triphosphate [ATP]) and 21 microg Ni L(-1) (decreased ATP). No significant changes were observed in whole-body total lipid, total protein, and lactate levels at any concentration. Whereas F1 neonates with mothers that were exposed to 21 microg Ni L(-1) showed increased resistance to acute Ni challenge, as measured by a significant (83%) increase in the acute (48-h) LC50, F1 neonates with mothers that were exposed to 42 microg Ni L(-1) were no more tolerant of acute Ni challenge than control animals were. Nickel accumulations in F1 animals chronically exposed to 21 and 42 microg Ni L(-1) were 11- and 18-fold, respectively, above control counterparts. The data presented suggest that chronic Ni exposure to two successive generations of D. magna lowered the overall energy state in the second generation. Whereas the quantity of neonates produced was not affected, the quality was; thus, environmentally meaningful criteria for regulating waterborne Ni concentrations in freshwater require consideration of possible multigenerational effects.

Nickel, lead, and cadmium induce differential cellular responses in sea urchin embryos by activating the synthesis of different HSP70s
Geraci, F. Pinsino, A. Turturici, G. Savona, R. Giudice, G. and Sconzo, G.
Biochem Biophys Res Commun. 2004: **322**(3): 873-877
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Treatment with heavy metals, such as nickel, lead or cadmium, elicits different cellular stress responses according to the metal used and the length of treatment. In Paracentrotus lividus embryos the inducible forms of HSP70 (HSP70/72) are different in molecular mass from the constitutively expressed HSP75, and they can be used as markers of cellular stress. Even a short treatment with each metal induces the synthesis of HSP70/72 which remain stable for at least 20h and differ little in their isoelectric points. Continuous treatment from fertilization with nickel or lead produces late irregular pluteus embryos, with peak HSP70/72 synthesis at blastula followed by the arrest of synthesis by pluteus. On the contrary, the same treatment with cadmium induces continuous HSP70/72 synthesis and produces irregular gastrula embryos which then degenerate. Moreover, a long treatment induces over control embryos a slight increase in the amount of constitutive HSP75 during development while lead treatment depresses constitutive HSP75 at early stages and doubles its quantity at late stages.

[Effects of T-type calcium channel blockers on spontaneous meiotic maturation of mouse ovarian oocytes in vitro].
Hotsuliak I., Berdyieva T. K. and Libert S. V.
Fiziol Zh. 2002;48(1):98-101.

We studied the effects of blockers of the T-type Ca2+ channels on resumption of meiosis and spontaneous meiotic maturation of murine ovarial oocytes in vitro. We found that the efficiency of suppression of oocyte maturation by blockers of the Ca2+ channels increases in the following succession: amiloride hydrochloride, mebifradil, NiCl2. It is supposed that there is a possibility to use blockers of the T-type Ca2+ channels for creation of new combined intrauterine contraceptives.

Rat ENaC expressed in Xenopus laevis oocytes is activated by cAMP and blocked by Ni(2+).
Segal A., Cucu D., Van D. W. and Weber W. M.
FEBS letters. 2002;515(1-3):177-83.

We used oocytes of the South African clawed toad Xenopus laevis to express the three subunits of the epithelial Na(+) channel from rat distal colon (rENaC). We combined

conventional dual-microelectrode voltage-clamp with continuous capacitance (C(m)) measurements and noise analysis to evaluate the effects of cAMP and Ni(2+) on rENaC. Control oocytes or rENaC-expressing oocytes exhibited no spontaneous fluctuations in current. However, in rENaC-expressing oocytes amiloride induced a marked plateau-shaped rise of the power density spectra. Recordings using four different concentrations of amiloride revealed that the blocker-channel interactions were of the first order. A cocktail of the membrane permeant cAMP analogue chlorophenylthio-cAMP and IBMX (cAMP cocktail) increased amiloride-sensitive current (I(ami)) and conductance (G(ami)). Furthermore, C(m) was also increased following cAMP application, indicating an increase in plasma membrane surface area. Noise analysis showed that cAMP increased the number of active channels in the oocyte membrane while single-channel current decreased. From these data we conclude that cAMP triggered exocytotic delivery of preformed rENaCs to the plasma membrane. Ni(2+) (2.5 mM) inhibited about 60% of the rENaC current and conductance while C(m) remained unaffected. Noise analysis revealed that this inhibition could be attributed to a decrease in the apparent channel density, while single-channel current did not change significantly. These observations argue for direct effects of Ni(2+) on channel activity rather than induction of endocytotic removal of active channels from the plasma membrane.

High molecular mass egg fucose sulfate polymer is required for opening both Ca2+ channels involved in triggering the sea urchin sperm acrosome reaction.
Hirohashi, N. and V. D. Vacquier
J Biol Chem. 2002: 277(2): 1182-1189.

A linear fucose sulfate polymer (FSP), > 10(6) daltons, is a major component of sea urchin egg jelly. FSP induces the sperm acrosome reaction (AR), an exocytotic process required for animal fertilization. Two Ca(2+) channels activate during AR induction, the first opens 1 s after FSP addition, and the second opens 5 s after the first. Mild acid hydrolysis of FSP results in a linear decrease in polymer size. The ability of FSP to induce the AR and activate sperm Ca(2+) channels decreases with increasing time of hydrolysis. Hydrolyzed FSP of approximately 60 kDa blocks intact FSP from inducing the AR. At 44 microg/ml hydrolyzed FSP, Ca(2+) entry into sperm is almost equal to that occurring in 3.8 microg/ml intact FSP; however the AR is not induced. The shape of the [Ca(2+)](i) increase curve and use of the Ca(2+) channel blockers nifidipine and Ni(2+) indicate that hydrolyzed FSP opens the second Ca(2+) channel, but not the first, and thus does not induce the AR. The giant size of intact FSP is required to open both Ca(2+) channels involved in triggering the AR.

Specific amino acids moderate the effects on Ni2+ on the testosterone production of mouse leydig cells in vitro.
Forgács Z., Némethy Z., Révész C. and Lázár P.
J Toxicol Environ Health Part A. 2001;62(5):349-58.

The purpose of this investigation was to study the effectiveness of two nickel-binding amino acids, histidine (His) and cysteine (Cys), to prevent the inhibitory action of Ni2+ on testosterone (T) production by mouse primary Leydig cell culture. The maximal human chorionic gonadotropin (hCG)-stimulated T response was measured by radioimmunoassay (RIA) in the culture media. Three types of experiments were performed. In a concentration-response study, Ni2+ (62.5 to 1,000 microM) was added to the cells simultaneously with equimolar or twice the equimolar concentrations of His or Cys and the cultures were maintained for 48 h. Nickel-induced reduction in T production was completely prevented by equimolar concentrations of His at Ni2+ concentrations of 125, 250, and 500 microM; equimolar or twice the equimolar concentrations of His were only partially effective at 1,000 microM Ni2+. Protective action of Cys was complete only at the lowest concentration of Ni2+ (125 microM). In a second series, the cells were incubated for various times (0.5 to 48 h) with 1,000 microM Ni2+ in the presence of 2,000 microM His or Cys. Increasing the time of incubation, the protective effect of both amino acids against Ni2+ was reduced. In a third series, attempts were made to reverse the action of 1,000 microM Ni2+ after incubation with cells for various times (0.5 to 24 h), followed by exposure to 2,000 microM His or Cys. Cell cultures were maintained for 48 h. A partial recovery of hCG-stimulated T production could be observed only if the amino acid was added not later than 4 h after the metal. This time was also required to elicit the T depression produced by Ni2+. Administration of either His or Cys at later times had no effect. Our results show that both His and Cys are able to moderate the effects of Ni2+ on Leydig cell T production, depending on the concentration of this metal ion, as well as on amino acid. However, at higher Ni2+ concentrations the complete protection by His or Cys is only temporary. Administration of these amino acids after the Ni2+-produced decrease in T production was not able to reverse the process.

Evaluation of nickel-zinc interactions by means of bioassays with amphibian embryos

Herkovits, J.,Pérez-Coll, C. S. and Herkovits, F. D
Ecotoxicol Environ Saf. 2000: 45(3): 266-273.

The nickel hazard was evaluated by means of a 7-day toxicity test with Bufo arenarum embryos. The LC(50) values for this metal from 24 to 168 h diminished from about 26 to 1.8 mg Ni(2+)/L, respectively, but from 96 h onward, the LC(50) varied very slightly. Although a noticeable difference among the LC(50) and LC(10) or LC(90) was observed at 24 h of exposure, these parameters tended to a similar value at 168 h of exposure while the confidence intervals of LC(50) overlapped all other confidence interval values. These results, plotted as toxicity profile curves, are useful for determining time and concentration thresholds for Ni. Nickel-zinc interactions on B. arenarum embryos were evaluated by means of simultaneous treatments with both cations (Ni: 5-35 mg Ni(2+)/L; Zn: 0.5-130 mg Zn(2+)/L). As a general pattern, low Zn concentrations (0.5 mg Zn(2+)/L) did not have a clear-cut effect on Ni toxicity, higher Zn concentrations (2-20 mg Zn(2+)/L) enhanced Ni toxicity, and concentrations of 30 mg Zn(2+)/L and higher had a beneficial effect in most cases. The metal interaction studies provide a scientific basis for the establishment of water quality criteria for wildlife protection purposes.

Toxicity of nickel to a soil-dwelling springtail, Folsomia fimetaria (Collembola: Isotomidae).
Scott-Fordsmand J. J., Krogh P. H. and Hopkin S. P.
Ecotoxicol Env Safety. 1999;43(1):57-61.

Exposure of the collembolan Folsomia fimetaria L. to nickel via soil caused significant mortality and reduced growth and reproductive output. Nickel may be present in elevated concentrations due to anthropogenic discharge. Although collembolans are very numerous and important organisms in the soil ecosystem, the effect of nickel has not previously been studied on these organisms. The aim of this study was to investigate the toxic effects of high soil nickel concentrations on the collembolan F. fimetaria following a 3-week exposure in a loamy sand spiked with nickel up to 1000 mg Ni/kg. A 10% decrease in adult female numbers at 427 mg Ni/kg and at 645 mg Ni/kg for adult male numbers was observed for nickel-spiked soil. Juvenile numbers were reduced at 701 mg Ni/kg following a 3-week exposure. The corresponding EC50 values were 786 mg Ni/kg for females, 922 mg Ni/kg for males, and 859 mg Ni/kg for juveniles. The reproductive output seems to be the most sensitive parameter being reduced at soil

nickel concentrations above 173 mg Ni/kg (EC10). Adult growth was not affected by soil nickel concentrations up to 1000 mg Ni/kg, but juvenile growth was reduced at concentrations above 480 mg Ni/kg (EC10).

Cadmium(II), unlike nickel(II), inhibits 8-oxo-dGTPase activity and increases 8-oxo-dG level in DNA of the rat testis, a target organ for cadmium(II) carcinogenesis.
Bialkowski K., Bialkowska A. and Kasprzak K. S.
Carcinogenesis. 1999;20(8):1621-4.

8-Oxo-2'-deoxyguanosine 5'-triphosphate pyrophosphohydrolase (8-oxo-dGTPase) is an enzyme which prevents incorporation into DNA of promutagenic 8-oxo-2'-deoxyguanosine (8-oxo-dG) from a deoxynucleotide pool damaged by endogenous oxidants. Its inhibition may thus be carcinogenic. We previously found that Cd(II) inhibited 8-oxo-dGTPase in both cell free systems and cultured cells. To verify this finding in a relevant animal model, we investigated the effects of Cd(II) on cellular 8-oxo-dGTPase activity and nuclear DNA 8-oxo-dG levels in the rat testis, a target organ for Cd(II) carcinogenesis. Ni(II), which does not induce testicular tumors in rats and is a weaker in vitro inhibitor of 8-oxo-dGTPase than Cd(II), was investigated as a comparison. Male F344/NCr rats were given a single s.c. dose of 20 micromol Cd(II) acetate, 90 micromol Ni(II) acetate or 180 micromol sodium acetate (controls) per kg body wt and killed 2, 8, 24 or 48 h later (three rats/time point). Cd(II) caused a gradual decrease in testicular 8-oxo-dGTPase activity with time. It became significant only after 8 h post-injection ($P < 0.05$) and resulted in a final 50% loss of the enzyme activity at 48 h ($P < 0. 01$). Although the results for Ni(II) at 8 h and later were apparently lower than the controls, the decrease did not reach statistical significance. Treatment of rats with Cd(II) led to an early and progressive increase (from 130% at 2 h to 200% at 48 h versus the controls) of the 8-oxo-dG level in testicular DNA ($P < 0. 05$ or better). Ni(II) acetate also tended to raise the testicular 8-oxo-dG level, but the increase was transient, with an apparent maximum at 8 h, and did not approach statistical significance ($P < 0. 2$). Thus, Cd(II), unlike Ni(II), is able to inhibit 8-oxo-dGTPase activity and to raise 8-oxo-dG levels in rat testicular DNA. However, the time course of both effects indicates that 8-oxo-dGTPase inhibition is most likely not the sole cause of the increase in 8-oxo-dG.

Non-cancer risk assessment for nickel compounds: issues associated with dose-response modeling of inhalation and oral exposures.
Haber L. T., Allen B. C. and Kimmel C. A.
Toxicol Sci. 1998;43(2):213-29.

This report presents the results of noncancer dose-response modeling for inhalation and oral exposures to nickel compounds using the NOAEL/LOAEL and benchmark dose (BMD) approaches. Several key issues associated with the implementation of the BMD approach were examined. Primary among them are difficulties associated with use of data for which the dose-response shape is poorly defined: nonuniqueness of maximum likelihood estimates and lower bounds equal to zero. In addition, several generalizable properties of the "hybrid approach" for modeling continuous endpoints were identified. A hybrid modeling approach allows one to consider "biological significance" on an individual (rather than group) basis; differences between individual- and group-based biological significance in the definition of benchmark response (BMR) levels are elucidated. In particular, it is shown that BMDs defined using group-based BMRs may be more like LOAELs than NOAELs. Application of cross-chemical and cross-endpoint comparisons suggest that, for chronic inhalation exposure, nickel sulfate appears to be as toxic or more toxic than nickel subsulfide and nickel oxide, although the high response rates for the latter two compounds at the lowest chronically administered concentration make such conclusions problematic. A nickel reference concentration could be derived based on the most sensitive benchmark concentration for chronic inhalation exposure to nickel sulfate, 1.7 x 10(-3) mg Ni/m3 for lung fibrosis in male rats. Analyses of oral studies of nickel sulfate and nickel chloride suggest that an appropriate basis for the nickel oral reference dose would be a BMD of 4-5 mg Ni/kg/day, based on increased prenatal mortality. (Uncertainty factors were not determined and neither an RfD nor an RfC was derived in this paper.) The BMD approach provides appropriate quantitative support for toxicological judgment; this paper addresses specific issues associated with the role of the BMD approach in noncancer risk assessment. Resolution of these and other issues may require the accumulation of a number of case studies such as the one presented here.

Ni2+ treatment causes cement gland formation in ectoderm explants of Xenopus laevis embryo.
Huang Y. and Ding X. Y.
Cell Res. 1999;9(1):71-6.

We found T-type calcium channel blocker Ni2+ can efficiently induce the formation of cement gland in Xenopus laevis animal cap explants. Another T-type specific calcium channel blocker Amiloride can also induce the formation of cement gland, while L-type specific calcium channel blocker Nifedipine has no inductive effect. These results may offer us an new approach to study the differentiation of cement gland through the change of intracelluar calcium concentration.

In vitro comparative effect of Cd2+, Ni2+, and Co2+ on mouse postblastocyst development.
Paksy K., Forgács Z. and Gáti I.
Environ Res. 1999;80(4):340-7.

Postblastocyst development of mouse preembryos was studied in vitro in order to determine direct effect of Cd2+, Ni2+, and Co2+ ions on embryogenesis during the peri-implantation stage. Uterine horns were flushed on Day 4 of pregnancy and expanded blastocysts were cultured for 4 days in the presence of micromolar Cd2+ (1.1-26.4), Ni2+ (0. 1-500) or Co2+ (1-200). Area of trophoblast outgrowth was measured and used as a quantitative toxicological endpoint. Hatching, attachment, outgrowth, and formation of inner cells mass were also registered. Significant adverse effect on the development stages were observed at 2.2 microM (Cd2+), at 10 microM (Ni2+), and at 100 microM (Co2+). Cd2+ and Co2+ decreased the area of trophoblast markedly at concentrations of 1.1 and 10 microM, respectively. Ni2+ exposure resulted in a slight increase at 10 microM followed by a marked reduction in the trophoblast area at 250 microM. Reduced proliferative ability of trophoblast cells may point to compromised invasiveness of the embryo. The lowest Cd2+ concentration (1.1 microM=0.25 microg/ml) significantly deteriorating trophoblast development was found to be lower than Cd levels ranging up to 0.512 microg/g, reported in clinical ovarian samples of occupationally nonexposed women. The morphological alteration and loss of cellular contacts in blastocysts induced by exposure to Cd2+, Ni2+, or Co2+ may adversely influence adhesion and recognition events and may disturb aggregation of mononuclear trophoblastic cells to multinucleated cells in the course of peri-implantation in vivo as well.

Effect of Ni2+ on the testosterone production of mouse primary Leydig cell culture.
Forgács Z., Paksy K., Lázár P. and Tátrai E.
J Toxicol Environ Health Part A. 1998;55(3):213-24.

This study evaluated the effects of Ni2 on testosterone (T) production of mouse Leydig cells in vitro following an in vivo or in vitro exposure. CFLP mice were subjected to repeated exposure (4 treatments, subcutaneously, every 3 d) to 10, 20 or 40 mg/kg body weight of NiSO4 or 1.0 ml of 0.9% NaCl solution. Depressed human chorionic gonadotropin (hCG)-stimulated T response was seen over a 48-h culture of testicular interstitial cells obtained from the animals exposed to 20 mg/kg or higher dose of NiSO4, while the basal T production remained unaltered. There were no Ni2+-related changes in the body weights or in the weights of testes, epididymides, adrenals, and kidneys. No histopathological alteration was found in the examined organs of NiSO4-treated groups except the dose-dependent tubular lesions in kidney as a result of a specific rather than a general cytotoxic action. To assess the direct effect of Ni2+ on Leydig-cell T production, testicular interstitial cells were cultured with Ni2+ (62.5 to 1000 microM) for 48 h in the presence or absence of maximally stimulating concentration of hCG. Dose-dependent depression in hCG-stimulated T production was seen at 125 microM or higher dose of Ni2+, while basal T production was unaffected. In order to evaluate the time dependency of this effect the cells were cultured for various times in the presence or absence of 250 and 1000 microM Ni2+. Decreased hCG-stimulated T production was found in the cultures maintained at least for 4 h in the presence of 1000 microM Ni2+, whereas at 250 microM at least 16 h was required to elicit the depression. Cell viability was assessed by a metabolic activity (MTT) assay. The viability of cells was unaltered by 250 microM Ni2+, and only a slight decrease was found even at the end of the 48-h culture period in the presence of 1000 microM Ni2+. Our results show a dose-related depression in stimulated T production of mouse Leydig cells in culture following either in vivo or in vitro Ni2+ treatment at a dose that does not induce any general toxic or significant cytotoxic action. The data of the time-course study indicate that the effect of Ni2+ on Leydig-cell T production is both time and concentration dependent, and not due to cytotoxicity.

Limited effects of heavy metal pollution on foraging and breeding success in the curlew (Numenius arquata).
Currie, D. and J. Valkama
Environ Pollut., 1998: 101(2): 253-261.

We examined the effect of heavy metal pollution on the foraging success and breeding performance of the curlew (Numenius arquata) along a documented pollution gradient from a point source, and also by comparing foraging and breeding parameters between the polluted site and two non-polluted areas. Prey biomass and abundance, and foraging success did not vary along the pollution gradient, and were no less at the polluted site than in the non-polluted areas. Furthermore, there was no differnece in adult weight during incubation between the polluted and non-polluted areas. There were also no differences in measures of breeding success along the pollution gradient, or between areas, which could be attributed to pollution per se. Egg shells from the polluted area had higher concentrations of heavy metals than in the non-polluted areas, and egg shells close to the pollution source were contaminated more than those further from it. However, there was no difference in calcium concentrations of egg shells or egg-shell thickness between areas. We conclude that in this study there were minimal immediate effects of heavy metal pollution on foraging and breeding success in the curlew.

Developmental abnormalities and DNA-protein crosslinks in sea urchin embryos exposed to three metals.
Garman G. D., Anderson S. L. and Cherr G. N.
Aquatic Toxicol. 1997;39(3-4):247-65.

Two sublethal responses were used to investigate the effects of genotoxic metals on embryos of the purple sea urchin, Strongylocentrotus purpuratus. In addition to the standard measurement of developmental success, we used a novel genotoxic response, DNA-protein crosslink (DPC) induction, to assess the effects of embryo exposure to pentavalent arsenate (As), nickel (Ni) and hexavalent chromate (Cr). The procedure for sea urchin embryo DPC measurement was adapted from a mammalian cell assay using potassium-SDS precipitation and a DNA fluorochrome to quantify relative amounts of free and protein-bound DNA. Developmental abnormality and DPCs increased after a 48-h exposure to each of the three metals. Lowest observable effect concentrations (LOECs) for development were 0.011 mg/L As, 0.40 mg/L Ni, and 2.5

mg/L Cr. LOECs calculated for the DPC response to these same three metals were 0.023, 8.0, and 10.0 mg/L, respectively. DPCs were transiently high in transcription of the embryonic genome. By the gastrula and prism stages (subsequent to embryo gene transcription), there was a significant decrease in DPCs. Ni-exposed embryos exhibited the greatest magnitude of adverse effect in embryos exposed through the blastula stage, as compared to those exposed from blastula through late gastrula stage. We hypothesize that stage-sensitivity to Ni in sea urchin embryos may be related to the induction of persistent DPCs, and the prevention of normal transcription of the embryonic genome.

Distribution of nickel in body fluids and organs of rats chronically exposed to nickel sulphate.
Severa J., Vyskocil A., Fiala Z. and Cizkova M.
Human Exp Toxicol. 1995;14(12):955-8.

1. Male and female rats were given 100 mg Ni L-1 (as nickel sulphate) in drinking water for 6 months. 2. The feeding of nickel was associated with an increased concentration of nickel in body fluids and organs. The highest concentrations of nickel were found in the liver of both male and female rats. In male rats nickel levels decreased in the order: liver > kidney = whole blood = serum > testes > urine. In female rats the decreasing order was similar: liver > kidney = whole blood = serum = plasma > urine > ovaries. 3. No significant differences were found between nickel concentrations in organs (except ovaries), blood and urine of rats exposed for 3 months and those exposed for 6 months indicating the reaching of a steady state of nickel in the rat during long-term exposure. 4. The urinary excretion of the orally administered nickel was only 2% of absorbed dose (supposing 1% Ni absorption).

Ni2+ inhibition induces asymmetry in axonemal functioning and bend initiation of bull sperm.
Lindemann C. B., Walker J. M. and Kanous K. S.
Cell Mot Cytosk. 1995;30(1):8-16.

Bull sperm extracted with 0.1% Triton X-100 can be reactivated to full motility with 0.33 mM Mg-ATP (sperm models). When motile sperm models are treated with 0.66 mM NiSO4, spontaneous motility is lost. During the transition to motility arrest, the beat becomes progressively more asymmetric, finally arresting at one extreme of the beat cycle. After spontaneous motility has been lost, the flagellum retains the ability to respond to mechanical stimulation. If a microprobe is used to bend the flagellum in the

direction opposite to its own prevailing curvature and released, the recoil is rapid and overshoots the equilibrium position. When the same flagellum is manipulated in the opposite direction (into a tighter bend of the existing curve), the recoil is slower and does not exceed the initial bend. If a microprobe is used to carefully bend the whole flagellum into a curve, the flagellum will resume continuous beating, but only if the imposed bend is in the direction opposite the natural curvature. The reinstated beating activity (mechanical reactivation) is sustained as long as the flagellum is held by the microprobe. The rate of change of the shear angle in these mechanically reactivated, Ni(2+)-inhibited sperm suggests an impaired rate of sliding on one side of the axoneme compared to similarly restrained control sperm. It appears that Ni2+ has a selective inhibitory effect on the dynein arms that bend the flagellum in one direction. Furthermore, the remaining functional arms activate only when the flagellum is bent in the direction opposing their own action.

Dynamics of thin filopodia during sea urchin gastrulation
Miller, J. Fraser, S. E. and McClay, D.
Development. 1995; 121(8): 2501-2511.

At gastrulation in the sea urchin embryo, a dramatic rearrangement of cells establishes the three germ layers of the organism. Experiments have revealed a number of cell interactions at this stage that transfer patterning information from cell to cell. Of particular significance, primary mesenchyme cells, which are responsible for production of the embryonic skeleton, have been shown to obtain extensive positional information from the embryonic ectoderm. In the present study, high resolution Nomarski imaging reveals the presence of very thin filopodia (02-0.4 micron in diameter) extending from primary mesenchyme cells as well as from ectodermal and secondary mesenchyme cells. These thin filopodia sometimes extend to more than 80 microns in length and show average growth and retraction rates of nearly 10 microns/minute. The filopodia are highly dynamic, rapidly changing from extension to resorption; frequently, the resorption changes to resumption of assembly. The behavior, location and timing of active thin filopodial movements does not correlate with cell locomotion; instead, there is a strong correlation suggesting their involvement in cell-cell interactions associated with signaling and patterning at gastrulation. Nickel-treatment, which is known to create a patterning defect in skeletogenesis due to alterations in the ectoderm, alters the normal position-dependent differences in the thin filopodia. The effect is present in recombinant embryos in which the ectoderm alone was treated with nickel, and is absent in recombinant embryos in which only the primary mesenchyme cells were treated, suggesting that the filopodial length is substratum dependent rather than being primary

mesenchyme cell autonomous. The thin filopodia provide a means by which cells can contact others several cell diameters away, suggesting that some of the signaling previously thought to be mediated by diffusible signals may instead by the result of direct receptor-ligand interactions between cell membranes.

Effects of teratogenic concentrations of Zn2+, Cd2+, Ni2+, Co2+, and Cu2+ in FETAX assays on metallothionein (MT) and MT-mRNA contents of Xenopus laevis embryos.
Sunderman F. W., Jr., Plowman M. C., Slaisova O., Grbac-Ivankovic S., Foglia L. and Crivello J. F.
Met Ions in Biol Med. 1994;3:17-22.

Xenopus laevis embryos were exposed to metals from 5 to 101 hours post-fertilization according to the FETAX (frog embryo teratogenesis assay: Xenopus) protocol. Control and metal-exposed embryos were assayed for metallothionein (MT) and MT-mRNA after each 24-hour interval. Metals were added to FETAX medium at concentrations (umol/L) that caused malformations in greater than 95% and mortality in less than 7% of embryos: Zn2+ (300); Cd2+ (18); Ni2+ (56); Co2+ (1,800); and Cu2+ (5.6). Embryos were analyzed for MT by silver-saturation and MT-mRNA by reverse transcriptase/polymerase chain reaction methods. In controls at 29, 53, 77, and 101 hours post-fertilization, median MT-mRNA contents were 7, 19, 33, and 34 copies x 10(6)/embryo, and median MT contents were 6, 11, 17, and 20 pmol/embryo, respectively. In Zn2+-exposed embryos at the same four intervals, median MT-mRNA contents were 10, 21400,* 158, and 1* copies x 10(6)/embryo, and median MT contents were 7, 19, 46,* and 78* pmol/embryo. In Cd2+-exposed embryos at the same four intervals, median MT-mRNA contents were 17, 4500,* 2020,* and 239 copies x 10(6)/embryo, and median MT contents were 6, 15, 30,* and 56* pmol/embryo (* p less than 0.05 vs controls). MT-mRNA and MT contents were unaffected in Ni2+-, Co2+-, or Cu2+-exposed embryos. This study shows that increased MT-mRNA and MT levels may be involved in Zn2+- and Cd2+-teratogenesis, but are unlikely to contribute to teratogenicity of Ni2+, Co2+, or Cu2+ in Xenopus embryos.

Specific amino acids modulate the embryotoxicity of nickel chloride and its transfer to the rat embryo in vitro.
Saillenfait A. M., Payan J. P., Sabate J. P., Langonne I., Fabry J. P. and Beydon D.
Toxicol Appl Pharma. 1993;123(2):299-308.

To investigate the effects of amino acids on the embryotoxicity and placental transfer of nickel chloride (NiCl2), Day 10 rat embryos were cultured in rat serum medium containing NiCl2 or 63NiCl2 (0.34 or 0.68 mM Ni), with or without L-histidine (2 mM), L-aspartic acid, glycine (2 or 8 mM), or L-cysteine (2 mM). After 26 hr, conceptuses were assessed for survival, growth and development, and malformations. The 63Ni contents of embryos and yolk sacs and the extent of 63Ni binding to the proteins of the culture medium were also determined. NiCl2 alone did not affect the embryonic development at 0.34 mM and caused growth retardation and brain and caudal abnormalities at 0.68 mM. Coincubation of L-histidine with 0.34 mM Ni increased Ni concentrations in embryonic tissues compared to 0.34 mM 63Ni alone, but did not elicit NiCl2 embryotoxicity. Coincubation of L-cysteine with 0.34 mM Ni elicited growth retardation and brain abnormalities caused by NiCl2 and increased yolk sac concentrations of 63Ni compared to 0.34 mM 63Ni alone. In contrast, coincubation of L-histidine, L-cysteine, or L-aspartic acid with 0.68 mM Ni reduced the growth retardation and the incidence and/or severity of brain defects caused by NiCl2 and decreased the concentrations of 63Ni in the yolk sacs, compared to 0.68 mM 63Ni alone. L-Histidine also reduced the percentage of NiCl2-elicited caudal defects. Coincubation with glycine did not NiCl2-elicited caudal defects. Coincubation with glycine did not affect the embryotoxic profile, nor the placental transfer of NiCl2. In the presence of L-histidine, L-cysteine, or L-aspartic acid, there was a shift of 63Ni binding from the high-molecular-weight proteins of the culture medium to the low-molecular-weight fraction. Thus, specific extracellular amino acids can modulate the embryotoxicity and placental transfer of NiCl2 in vitro. The pattern of this modulation is dependent on the concentration of NiCl2, as well as on the amino acid.

Block of current through T-type calcium channels by trivalent metal cations and nickel in neural rat and human cells.
Mlinar B. and Enyeart J. J.
The J Physiol. 1993;469:639-52.

1. The effects of the trivalent cations yttrium (Y3+), lanthanum (La3+), cerium (Ce3+), neodymium (Nd3+), gadolinium (Gd3+), holmium (Ho3+), erbium (Er3+), ytterbium (Yb3+) and the divalent cation nickel (Ni2+) on the T-type voltage gated calcium

channel (VGCC) were characterized by the whole-cell patch clamp technique using rat and human thyroid C cell lines. 2. All the metal cations (M3+) studied, blocked current through T-type VGCC (IT) in a concentration-dependent manner. Smaller trivalents were the best T-channel antagonists and potency varied inversely with ionic radii for the larger M3+ ions. Estimation of half-maximal blocking concentrations (IC50s) for IT carried by 10 mM Ca2+ resulted in the following potency sequence: Ho3+ (IC50 = 0.107 microM) approximately Y3+ (0.117) approximately Yb3+ (0.124) > or = Er3+ (0.153) > Gd3+ (0.267) > Nd3+ (0.429) > Ce3+ (0.728) > La3+ (1.015) > > Ni2+ (5.65). 3. Tail current measurements and conditioning protocols were used to study the influence of membrane voltage on the potency of these antagonists. Block of IT by Ni2+, Y3+, La3+ and the lanthanides was voltage independent in the range from -200 to +80 mV. In addition, the antagonists did not affect macroscopic inactivation and deactivation of T-type VGCC. 4. Increasing the extracellular Ca2+ concentration reduced the potency of IT block by Ho3+, indicative of competitive antagonism between this blocker and the permeant ion for a binding site. 5. The results suggest that the mechanism of metal cation block of T-type VGCC is occlusion of the channel pore by the antagonist binding to a Ca2+/M3+ binding site, located out of the membrane electric field. 6. Block of T-type VGCC by Y3+, lanthanides and La3+ differ from the inhibition of high voltage-activated VGCC block in several respects: smaller cations are more potent IT antagonists; block is voltage independent and the antagonists do not permeate T-type channels. These differences suggest corresponding structural dissimilarities in the permeation pathways of low and high voltage-activated Ca2+ channels.

Evaluation of the toxicity of different metal compounds in the developing brain using aggregating cell cultures as a model.
Monnet-Tschudi F., Zurich M. G. and Honegger P.
Toxicol In Vitro. 1993;7(4):335-9.

To evaluate their toxicity in the developing brain, eight metal compounds, (bismuth sodium tartrate (BiNa-tartrate), CdCl2, CoCl2, HgCl2, dimethylmercury, NiCl2, TlCl and triethyltin chloride (TET)) were tested in aggregating cell cultures of foetal rat telencephalon. The compounds were applied to the cultures continuously, either during an early developmental stage (between days 5 and 14) or during an advanced stage of maturation (between days 24 and 34). Changes in the activities of cell type-specific enzymes were used as a criterion for toxicity. A general cytotoxic effect was observed after treatment with either CdCl2, HgCl2 or TET at 10(-6) M, and with TlCl at 10(-5) M. Selective effects were found with BiNa-tartrate and dimethylmercury. CoCl2 did not modify the parameters tested, whereas a stimulant effect was found with NiCl2. The

effects of several compounds were development dependent: HgCl2, TET and TlCl were more toxic in immature cultures, whereas BiNa-tartrate, dimethylmercury and NiCl2 were more effective in differentiated cultures.

Inhibition of microtubule sliding by Ni2+ and Cd2+: evidence for a differential response of certain microtubule pairs within the bovine sperm axoneme.
Kanous K. S., Casey C. and Lindemann C. B.
Cell Mot Cytosk. 1993;26(1):66-76.

Bovine sperm, extracted with 0.1% Triton X-100, frozen at -20 degrees C for 48-120 hours, and thawed, disintegrated by microtubule sliding when 1 mM MgATP was added. Microtubules and outer dense fibers (ODFs) were usually extruded in groups or "bundles". A total of 44.5% of the cells extruded two distinct bundles, one from each side of the connecting piece, exhibiting opposite curvatures. Only one bundle was observed in 46.2% of the cells, and 9.2% showed no signs of sliding. Transmission electron microscopy (T.E.M.) showed one group consisting of the 4,5-6,7 elements, with the 9,1,2 elements on the other side of the axoneme making up the other bundle. T.E.M. revealed that when only one side of the axoneme had extruded elements, they were always from the 4,5-6,7 group. The remainder of the axoneme (8,9,1,2,3 and the central pair) was left relatively intact, suggesting a difference in the sliding response of the nine pairs of axonemal microtubules. These results indicate a predisposition for sliding between elements 7 and 8 over that between doublets 2 and 3, perhaps due to a disparity in activation thresholds. Also, both Ni2+ and Cd2+ appear to selectively block activation of 2-3 interdoublet sliding. Incubation with 0.25 mM Ni2+ prior to adding MgATP modified the percentages of sliding patterns: 8.6% demonstrated two-sided extrusion, 58.2% showed one-sided, and 33.2% had no extruded bundles. Again, when half the axoneme was missing, it was always the 4,5-6,7 group. Ten micromolar Cd2+ altered the sliding pattern similarly to Ni2+, with 28% two-sided extrusion, 55.9% one-sided extrusion and 16.1% with no extruded bundles. Either pretreatment regimen impeded extrusion of the 9,1,2 group in a high percentage of cells, compared to untreated cells. This specific inhibition of the 9,1,2 side by Ni2+ or Cd2+ is especially significant since Ni2+ also inhibits spontaneous wave initiation in bull sperm (Lindemann et al.: Journal of Cell Biology 87:420-426, 1980), and both Ni2+ and Cd2+ reportedly block the flagellar Ca(2+)-response in rat sperm (Lindemann and Goltz: Cell Motility and the Cytoskeleton 10:420-431, 1988; Lindemann et al.: Cell Motility and the Cytoskeleton 20:316-324, 1991).

Mg(2+)-deprivation enhances and Mg(2+)-supplementation diminishes the embryotoxic and teratogenic effects of Ni2+, Co2+, Zn2+, and Cd2+ for frog embryos in the FETAX assay.

Luo S. Q., Plowman M. C., Hopfer S. M. and Sunderman F. W., Jr.
A Clin Lab Sci. 1993;23(2):121-9.

The influence of Mg2+ on the embryotoxicity and teratogenicity of Ni2+, Co2+, Zn2+, and Cd2+ for Xenopus embryos was studied by an adaptation of the FETAX protocol. In seven assays, 25 groups of embryos were grown from 5 to 101 hours post-fertilization in FETAX media that contained five graded MgCl2 concentrations (0, 6.2, 62, 620, or 6,200 mumol per L), with or without added NiCl2 (56 mumol per L), CoCl2 (1,800 mumol per L), ZnCl2 (300 mumol per L), or CdCl2 (18 mumol per L). In FETAX assays performed with the standard Mg2+ concentration (620 mumol per L), the incidence of malformations in control embryos averaged 5.4 (SD +/- 1.3) percent; the incidence of malformations in the controls was increased at low Mg2+ concentrations (32 +/- 7 percent at 62 mumol per L; 100 percent at greater than or equal to 6.2 mumol per L). The specified additions of Ni2+, Co2+, Zn2+, or Cd2+ caused death in < 10 under standard conditions (620 mumol Mg2+ per L). Mg(2+)-deprivation greatly enhanced and Mg(2+)-supplementation significantly reduced the incidence and severity of the teratogenic and embryotoxic effects of Ni2+, Co2+, Zn2+, and Cd2+ (p the authors speculate that Mg2+ competes with the other divalent metal ions for a carrier mechanism involved in metal absorption or cellular uptake, or for binding to critical molecular targets.

Cell-cell interactions regulate skeleton formation in the sea urchin embryo
Armstrong, N. Hardin, J. and McClay, D. R.
Development. 1993 119(3): 833-840.

In the sea urchin embryo, the primary mesenchyme cells (PMCs) make extensive contact with the ectoderm of the blastula wall. This contact is shown to influence production of the larval skeleton by the PMCs. A previous observation showed that treatment of embryos with NiCl2 can alter spicule number and skeletal pattern (Hardin et al. (1992) Development, 116, 671-685). Here, to explore the tissue sensitivity to NiCl2, experiments recombined normal or NiCl2-treated PMCs with either normal or NiCl2-treated PMC-less host embryos. We find that NiCl2 alters skeleton production by influencing the ectoderm of the blastula wall with which the PMCs interact. The ectoderm is responsible for specifying the number of spicules made by the PMCs. In

addition, experiments examining skeleton production in vitro and in half- and quarter-sized embryos shows that cell interactions also influence skeleton size. PMCs grown in vitro away from interactions with the rest of the embryo, can produce larger spicules than in vivo. Thus, the epithelium of the blastula wall appears to provide spatial and scalar information that regulates skeleton production by the PMCs.

Embryotoxicity and teratogenicity of Ni2+ and Co2+ in Xenopus laevis.
Sunderman F. W., Jr.
Metal Compounds in Environment and Life: Interrelation between Chemistry and Biology. 1992;4:467-74.

Nickel chloride (NiCl2) and cobalt chloride (CoCl2) were tested by the FETAX (Frog Embryo Teratogenesis Assay: Xenopus) procedure. The median embryolethal concentrations (LC50) of Ni2+ and Co2+ were 0.37 and 10.4 mmol L-1. The median teratogenic concentrations (EC50) of Ni2+ and Co2+ were 2.5 and 25 umol L-1. The metals were potent teratogens in Xenopus, causing concentration-related increases of ocular, skeletal, gut, facial, and cardiac anomalies.

Nickel(II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis.
Kasprzak K. S., Diwan B. A., Rice J. M., Misra M., Riggs C. W., Olinski R. and Dizdaroglu M.
Chem Res Toxicol. 1992;5(6):809-15.

DNA base damage was studied in renal and hepatic chromatin of nickel(II)-injected pregnant female F344/NCr rats and their fetuses under conditions leading to initiation of sodium barbital-promotable renal tumors, but not liver tumors, in the male offspring. Pregnant rats were given a total of 90 or 180 mumol of nickel(II) acetate/kg body wt in a single ip dose on day 17 or in 2 or 4 ip doses between days 12 and 18 of gestation. Control rats received 180 mumol of sodium acetate/kg body wt. The animals were killed 24 or 48 h after the last injection. Chromatin was isolated from livers and kidneys from both adults and fetuses and analyzed by gas chromatography/mass spectrometry with selected ion monitoring. Eleven products derived from the purine and pyrimidine bases in DNA bases were identified and quantified. These were the following: 5-hydroxy-5-methylhydantoin, 5-hydroxyhydantoin, 5-(hydroxymethyl)uracil, cytosine glycol, thymine glycol, 5,6-dihydroxycystosine, 4,6-diamino-5-formamidopyrimidine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8-hydroxyadenine, 2-hydroxyadenine, and 8-hydroxyguanine (8-OH-Gua). Nickel(II) exposure increased the content of these

products, especially those derived from purines, in both renal and hepatic chromatin of pregnant rats. The major difference between these two organs was the content of 8-OH-Gua, which increased greatly in the kidney but remained unchanged in the liver.(ABSTRACT TRUNCATED AT 250 WORDS)

Commitment along the dorsoventral axis of the sea urchin embryo is altered in response to NiCl2.
Hardin, J., Coffman, J. A. Black, S. D and McClay, D. R.
Development 1992. 116(3): 671-685.

Few treatments are known that perturb the dorsoventral axis of the sea urchin embryo. We report here that the dorsoventral polarity of the sea urchin embryo can be disrupted by treatment of embryos with NiCl2. Lytechinus variegatus embryos treated with 0.5 mM NiCl2 from fertilization until the early gastrula stage appear morphologically normal until the midgastrula stage, when they fail to acquire the overt dorsoventral polarity characteristic of untreated siblings. The ectoderm of normal embryos possesses two ventrolateral thickenings just above the vegetal plate region. In nickel-treated embryos, these become expanded as a circumferential belt around the vegetal plate. The ectoderm just ventral to the animal pole normally invaginates to form a stomodeum, which then fuses with the tip of the archenteron to produce the mouth. In nickel-treated embryos, the stomodeal invagination is expanded to become a circumferential constriction, and it eventually pinches off as the tip of the archenteron fuses with it to produce a mouth. Primary mesenchyme cells form a ring in the lateral ectoderm, but as many as a dozen spicule rudiments can form in a radial pattern. Dorsoventral differentiation of ectodermal tissues is profoundly perturbed: nickel-treated embryos underexpress transcripts of the dorsal (aboral) gene LvS1, they overexpress the ventral (oral) ectodermal gene product, EctoV, and the ciliated band is shifted to the vegetal margin of the embryo. Although some dorsoventral abnormalities are observed, animal-vegetal differentiation of the archenteron and associated structures seems largely normal, based on the localization of region-specific gene products. Gross differentiation of primary mesenchyme cells seems unaffected, since nickel-treated embryos possess the normal number of these cells. Furthermore, when all primary mesenchyme cells are removed from nickel-treated embryos, some secondary mesenchyme cells undergo the process of "conversion" (Ettensohn, C. A. and McClay, D. R. (1988) Dev. Biol. 125, 396-409), migrating to sites where the larval skeleton would ordinarily form and subsequently producing spicule rudiments. However, the skeletal pattern formed by the converted cells is completely radialized. Our data suggest that a major effect of NiCl2 is

to alter commitment of ectodermal cells along the dorsoventral axis. Among the consequences appears to be a disruption of pattern formation by mesenchyme cells.

Nickel chloride teratogenesis in cultured rat embryos.
Saillenfait A. M., Sabate J. P., Langonne I. and de C. J.
Toxicol In Vitro. 1991;5(1):83-9.

Day 10 rat embryos were cultured in rat serum in the presence of 20-80 ug Ni as nickel chloride (NiCl2)/mL of culture medium, or in serum taken from rats, on day 10 of pregnancy, 1 hr after ip injection of 4 mg Ni/kg body weight (as NiCl2). Embryos were exposed to these mediums either for 26 or for 4 hr, and were then transferred to fresh serum for the remainder of the 26-hr culture period. Normal development was observed in embryos cultured in serum from treated females (which was found to contain about 17 ug Ni and 3.4 mg glucose/mL) or in 20 ug Ni/mL (as NiCl2) added directly to the culture medium. Some embryos were killed by exposure to 80 or 40 (or more) ug Ni/mL for 4 or 26 hr, respectively. Regardless of the duration of exposure, malformations appeared at 30 ug Ni/mL primarily in the cephalic region. Reduced caudal neural tube and branchial arches, and dilated optic vesicles were observed in embryos exposed to 40 ug Ni/mL for 26 hr. High incidences of poor yolk-sac circulation and incomplete turning, and significant decreases in yolk-sac diameter and number of somite pairs were observed in embryos exposed to 60 or 70 ug Ni/mL for 4 hr, or to 30 to 40 ug Ni/mL for 26 hr. Our results indicate that the early maternal blood consequences of a single ip injection of NiCl2 in mid-gestation are harmless to the development of day 10 cultured embryos and that nickel is embryotoxic in vitro at concentrations that are probably not reached in vivo under these maternal treatment conditions.

Teratogenicity of Ni2+ in Xenopus laevis, assayed by the FETAX procedure.
Hopfer S. M., Plowman M. C., Sweeney K. R., Bantle J. A. and Sunderman F. W., Jr.
Bio Trace Elem Res. 1991;29(3):203-16.

The teratogenicity of Ni2+ was tested by the FETAX (Frog Embryo Teratogenesis Assay: Xenopus) procedure in the South African frog, Xenopus laevis. In seven assays, beginning at 5 h postfertilization, groups of Xenopus embryos were incubated for 96 h in media that contained Ni2+ (added as NiCl2) at concentrations ranging from 1 x 10(-7) to 3 x 10(-3) mol/L; control groups were incubated in the same medium without added NiCl2. At 101 h postfertilization, surviving embryos were counted, fixed in formalin, and examined by microscopy to determine their developmental stages, malformations, and head-to-tail lengths. In control embryos, survival was greater than or equal to 95% and

malformations were less than or equal to 7%. Malformations were found in greater than 95% of embryos exposed to Ni2+ concentrations greater than or equal to 5.6 mumol/L. The most frequent malformations in Ni(2+)-exposed embryos were ocular, skeletal, and intestinal deformities; less common malformations included facial, cardiac, and integumentary deformities. Other abnormalities, not categorized as malformations, included stunted growth, dermal hypopigmentation, and coelomic effusions or hemorrhages. The median embryolethal concentration (LC50) of Ni2+ was 365 (SE +/- 9) mumol/L; the median teratogenic concentration (EC50) was 2.5 (SE +/- 0.1) mumol/L; the Teratogenic Index (TI = LC50/EC50) was 147 (SE +/- 5), indicating that Ni2+ is a potent teratogen for Xenopus laevis. Experiments in which Ni(2+)-exposures were limited to specific 24 h periods showed that Xenopus embryos were most susceptible to Ni(2+)-induced malformations on the second and third days of life, during the most active period of organogenesis.

A comparison of the sensitivity of three Daphnia magna populations under chronic heavy metal stress.
Munzinger A. and Monicelli F.
Ecotoxicol Environ Safety. 1991;22(1):24-31.

The results from chromium, nickel, and zinc bioassays with three populations of Daphnia magna indicate that the investigated strains are differently sensitive. Sublethal concentrations which have a negative effect on survival and reproduction after 21 days of exposure also cause a significant reduction in the body length and brood size of primiparous females. Problems and consequences of the repeatability of such bioassays between laboratories in different countries are discussed.

Effects of nickel on Daphnia magna during chronic exposure and alterations in the toxicity to generations pre-exposed to nickel.
Mànzinger A.
Water Res. 1990;24(7):845-52.

Daphnia magna was cultured at sublethal nickel concentrations. The mean life expectancy was significantly less when exposed to greater than or equal to 40 ppb Ni. Statistically significant reductions of the number of offspring, maximum body length of adults and of neonates were caused by greater than or equal to 80 ppb Ni. The length and brood size of primiparous animals were significantly less when exposed to greater than or equal to 120 ppb Ni. The intrinsic rate of population growth (r) was inversely proportional to nickel concentrations. During seven successive generations exposed to

160 ppb Ni, both the mean life span and the length of primiparous animals decreased significantly with increasing time. The progeny of nickel pre-exposed generations exhibited no adaptation towards nickel except an altered reproduction pattern which induced an increase of r. After the transfer into nickel-free water, the progeny of nickel pre-exposed generations showed an increase of body length, mean life span, number of offspring, brood size and brood number as well as maximum body length of neonates. However, the progeny of pre-exposed generations remained smaller than the progeny of an untreated generation.

Morphological transformation and catalase activity of Syrian hamster embryo cells treated with hepatic peroxisome proliferators, TPA and nickel sulphate.
Mikalsen S. O., Holen I. and Sanner T.
Cell Bio Toxicol. 1990;6(1):1-13.

The abilities of the hepatic peroxisome proliferators (HPPs) clofibrate, di(2-ethylhexyl)phthalate (DEHP), mono(2-ethylhexyl)-phthalate (MEHP), 2,4-dichlorophenoxy acetic acid (2,4-D), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) and tiadenol to induce morphological transformation and to increase the catalase activity of Syrian hamster embryo (SHE) cells were studied. DEHP, MEHP, clofibrate and tiadenol induced morphological transformation of SHE cells and increased the catalase activity. DEHP was more potent than clofibrate and tiadenol in both inducing catalase and morphological transformation, while MEHP seemed more potent than DEHP in inducing catalase, but not morphological transformation, 2,4,5-T and 2,4-D did not induce morphological transformation, but 2,4,5-T was more potent than clofibrate in increasing the catalase activity. These results show that several HPPs induce morphological transformation of SHE cells and an increase in the catalase activity. There is, however, no direct connection between these two parameters, as seen from the results of 2,4,5-T. The tumor promoter TPA, and the metal salt nickel sulphate, induced morphological transformation of SHE cells without any appreciable increase in the catalase activity. These results further corroborate the dissociation between induction of morphological transformation and the increase in catalase activity.

Drosophila melanogaster embryo cultures: an in vitro teratogen assay.
Bournias-Vardiabasis N.
Altern Lab Anim. 1990;18:291-300.

An in vitro teratogen assay has been developed that uses Drosophila embryo cell cultures. The endpoints selected in assessing the teratogenic potential of any agent

(physical or chemical) involves detection of interference with normal muscle and/or neuron differentiation, induction of heat shock (stress) proteins, and inhibition of normal neurotransmitter levels. Current studies involve use of reporter gene technology (protein fusions) to identify teratogenicity. Results so far suggest that the Drosophila assay is capable of accurately establishing if a particular agent tested can act as a teratogen by a variety of appropriate endpoints (morphological, biochemical, molecular). Furthermore, this assay can be used not only as a teratogen screen, but also in mechanistic studies of abnormal development, gene involvement in teratogenic resistance, and the possible role of heat shock proteins in preventing birth defects.

Uptake and release of 63Ni2+ by Xenopus embryos during early cleavage stages. Sunderman F. W., Jr., Mongillo F. J., Plowman M. C. and Brennan S. M. Biology of metals. 1990;2(4):214-8.

Uptake and release of 63Ni was studied in dejellied Xenopus laevis embryos exposed to 63Ni2+ (0.3-30 mumol/l) for 0.5-h intervals during the period 1-4.5 h post-fertilization (i.e. from first cleavage to early blastula stage). At first cleavage, the mean uptake of 63Ni by embryos was 12-17 times that by non-fertilized eggs, suggesting that conversion of the vitelline envelope to the fertilization envelope enhanced integumental permeability to 63Ni2+. 63Ni uptake by embryos at the 1-2-cell stage averaged 1.8-2.5 times that at the early blastula stage. An average of 5% of total 63Ni in washed embryos was recovered in isolated fertilization envelopes, indicating that 63Ni2+ passed through the envelope into internal compartments. Progressive increases of 63Ni uptake were seen with increasing exposure levels; after exposure during 1-1.5 h post-fertilization to the highest concentration of 63Ni2+ (30 mumol/l), 63Ni uptake averaged 11.4 (SD +/- 5.1) pmol/embryo. Rapid efflux of 63Ni was noted after 63Ni2(+)-exposed embryos were transferred to nickel-free medium; mean 63Ni contents at 0.25 h and 2 h post-exposure diminished to 50% and 15% of the initial values, regardless of the exposure level. The finding that Xenopus embryos are permeable to 63Ni2+ during early cleavage stages provides a convenient experimental system to investigate the embryotoxicity and teratogenicity of nickel.

Bioaccumulation of metals from a nickel smelter waste in P and F1 generations of exposed animals. II. Modulation of immune processes.
Reichrtov E., Oravec C., P len¡kov O., Horv thov J., Tak c L. and Bencko V.
J Hyg Epi Microbio Immun. 1989;33(3):245-51.

A group of female Chinchilla rabbits was exposed through inhalation to the metal aerosol derived from dumped waste of a nickel smelter. The experiments were carried out in a field exposure station. Increased levels of tissue immune complexes were found in the myocardium and lungs of P females, whereas F1 rabbits (exposed both prenatally and 6 weeks postnatally) from the same group of P females had significantly elevated serum circulating immune complexes as compared to controls. In P rabbits, nonspecific serum tumoricidal activity was increased by 8.2%, while in F1 animals the increase was by 14%. Transplantation immunity was examined in a group of inbred Lewis rats following the transplantation of a skin allograft from the ear of inbred Berlin-Druckrey rats. The mean time of allograft survival in animals following i.v. administration metal dust suspension 2 days prior to transplantation, was prolonged as compared to controls. On day 22 after allograft transplantation, lactate dehydrogenase activity was found to be reduced in peripheral lymphocytes, and the liver and spleen weight proved to be diminished. These findings suggest a modulating effect of the metal dust from a nickel smelter regarding nonspecific serum tumoricidal activity and transplantation immunity as well as immune complex formation.

Pathway of nickel uptake influences its interaction with heterochromatic DNA.
Sen P. and Costa M.
Toxicolo Appl Pharma. 1986;84(2):278-85.

Exposure of intact Chinese hamster ovary cells to water-soluble NiCl2 and to particulate crystalline NiS induced a concentration-dependent incidence of chromosomal aberrations which included gaps, breaks, and exchanges. Exposure of cells to crystalline NiS particles caused a high incidence of chromatid exchanges and dicentrics and produced what appears to be an effect on the condensation state of the heterochromatic long arm of the X chromosome. Treatment of cells with NiCl2 did not cause any significant effect on the long arm of the X chromosome, and there was a much lower incidence of the dicentric type of chromosomal aberrations compared to NiS. To examine whether the fragmentation/decondensation of the long arm of the X chromosome produced by crystalline NiS particles was due to a phagocytic pathway of uptake of NiS particles, cells were treated with NiCl2-albumin complexes that had been encapsulated in liposomes. Although treatment of cells with NiCl2-albumin complexes

yielded higher intracellular nickel levels than were obtained by treatment of cells with NiCl2, at comparable intracellular levels fragmentation/decondensation of the heterochromatic long arm of the X chromosome was observed when nickel (II) was delivered by way of a liposome but not when cells were treated with unencapsulated NiCl2. Ionic nickel alone irrespective of its delivery mechanism exhibited some preference for heterochromatin, since there was a higher incidence of aberrations observed in the heterochromatic centromeric region of chromosomes. These observations suggest that the pathway of delivery of Ni2+ from NiS particles may be responsible for a preferential interaction of this metal with heterochromatin leading to an effect on the condensation state/fragmentation of the heterochromatic long arm of the X chromosome.

Effect of simultaneous exposure to nickel chloride and benzo(a)pyrene on developing chick embryos.
Anwer J. and Mehrotra N. K.
Drug Chem Toxicology. 1986;9(2):171-83.

In the present investigation the effect of Benzo(a)pyrene (BP) and Nickel chloride, quite often identified in crude and refined or waste oil, when injected in combination, was investigated on developing chick embryos after exposing them through yolk sac route on 6th day of incubation. Exposure to this combination in different doses resulted in no new deformities other than those experienced in BP treated chick embryos. The mortality and malformations experienced in the chick embryos exposed to BP alone and NiCl2 alone were seen to be added when both the chemicals were simultaneously inoculated into the same chick eggs.

Recovery of mouse embryos after short-term in vitro exposure to toxic nickel chloride.
Storeng R. and Jonsen J.
Toxicol Letters. 1984;20(1):85-91.

The development of preimplantation mouse embryos in vitro was adversely affected by the addition of nickel chloride (NiCl2 X 6H2O) to the culture medium. For day 3 (4-8 cell) embryos developmental cessation occurred after 48 h in culture, in NiCl2 X 6H2O-containing medium. However, transfer to NiCl2 X 6H2O-free medium after 5 min, 1 h, and 3 h exposure, resulted in regaining of the developmental capacity for a proportion of the exposed embryos. The in vivo development, in pseudopregnant recipients, of in vitro nickel-exposed embryos was not significantly different from that in control embryos. The

results indicated that the effect of NiCl2 X 6H2O on the development of day 3 mouse embryos in vitro was reversible after a short exposure period.

[Embryo-toxic effects of nickel sulfate in Paracentrotus lividus: preliminary observations].
Deiana L., Congiu A. M. and Arena N.
Boll Soc Ital Biol Sper. 1983;59(11):1718-24.

A primary objective of the work described here was to determine the embryo-toxic effects induced by Nickel Sulfate upon the development of the Paracentrotus Lividus. Different concentrations of Nickel Sulfate have been dissolved in containers with not foul sea-water. The fecundation took place in the containers and verified by constant observations with a light microscope. The development of the Paracentrotus 1. was totally blocked at a concentration of 0.5 M.10(-2) of Nickel Sulfate, while an enormous development was observed at concentrations of 0.5 M.10(-3) and 0.5 M.10(-4). Finally, a concentration of 0.5 M.10(-5) caused a slow development. A normal development was observed only at a concentration of 0.5 M.10(-6). Our results support various hypotheses on the mechanism of action of the Nickel Sulfate as an inhibitor of the normal development.

Application of the in vitro embryo culture to the study of the mutagenic effects of nickel in male germ cells.
Jacquet P. and Mayence A.
Toxicol Letters. 1982;11(1-2):193-7.

In vitro embryo cultures were utilized to determine the mechanism of preimplantation loss of embryos derived from matings 3 and 4 weeks after treatment of male Balb/c mice with 56 mg/kg nickel nitrate. Treated and control males were allowed to mate with superovulated females weekly for 5 weeks following treatment, and the number of cleaved eggs as well as development of embryos to blastocysts and implantation were determined. Controls included also males treated with a dose of 40 mg/kg nickel nitrate which previously had been shown to be ineffective in the dominant lethal test. Fertilizing capacity of the spermatozoa as well as development of the cultured embryos were not influenced by a dose of 40 mg/kg nickel nitrate. A dose of 56 mg/kg significantly reduced, the fertilization rate 3 and 4 weeks after treatment but did not affect development of 2-cell embryos. These results demonstrate that the preimplantation loss induced by nickel treatment of males is due to toxic effect on spermatids and spermatogonia and not to a clastogenic action.

Effects of dietary nickel on mallards.
Eastin W. C., Jr. and O'Shea T. J.
J Toxicol Environ Health. 1981;7(6):883-92.

Thirty breeding pairs of mallards (Anas platyrhynchos) were randomly assigned to one of five treatment groups and were fed breeder mash containing 0, 12.5, 50.0, 200.0, or 800.0 ppm Ni (as the sulfate) for 90 d. Ni ingestion had no effect on egg production, hatchability, or survival of ducklings. After 90 d birds were bled, sacrificed, and necropsied. There were no significant differences in hematocrit; concentrations of hemoglobin, plasma triglyceride, and cholesterol; of plasma activities of ornithine carbamoyltransferase and alanine aminotransferase. A black tarry feces was noted in the high Ni dose group at necropsy, but no gross or histopathologic lesions were observed. Although absolute concentrations of Ni in tissues were low, there were significant accumulations in kidneys of birds fed Ni at all dietary levels and in feathers, blood, and livers of birds fed high doses of Ni compared with controls.

Effect of nickel chloride and cadmium acetate on the development of preimplantation mouse embryos in vitro.
Storeng R. and Jonsen J.
Toxicology. 1980;17(2):183-7.

Preimplantation mouse embryos were used to investigate the toxic effect of nickel chloride and cadmium acetate on early embryo development in vitro. Embryos at the 2- and 4-8 cell stage were cultured in approximately 0.05 ml of mouse embryo culture medium (No. 16), overlaid with paraffin oil and incubated in a humidified atmosphere of 5% CO2 in air for 48 h. NiCl2 . 6H2O was added to the culture medium at concentrations of 10-1000 microM, Cd(CH3COO)2 . 2H2O at concentrations of 10-50 microM. Morphological criteria were used to check embryonic development. Ten micromolars of nickel chloride affected adversely the development of Day 2 embryos (2-cell stage), whereas 300 microM was needed to affect Day 3 embryos (8-cell stage). Toxic effect of cadmium acetate on Day 2 embryos was observed at a concentration of 10 microM.

Effects of NiCl2 and NiO in Wistar rats after oral uptake and inhalation exposure respectively.
Weischer C. H., K”rdel W. and Hochrainer D.
Zentralbl Bakteriol Mikrobiol Hyg B. 1980;171(4-5):336-51.

The effects of NiCl2 and NiO after oral uptake and after inhalation exposure respectively were investigated in three experiments, using clinical and clinico-chemical methods. I. Oral application of NiCl2 in male rats over a period of 28 days. The NiCl2 concentrations were 2.5; 5.0 and 10.0 microgram/ml in drinking water. II. Inhalation exposure of male rats with NiO-aerosols (0.2; 0.4 and 0.8 mg/m3) over a period of 28 days. III. Inhalation exposure of non-pregnant and pregnant rats with NiO-aerosols (0.8; 1.6 and 3.2 mg/m3) over a period of 21 days. After oral application of NiCl2 and inhalation exposure of NiO in male rats a significant dose-dependent hyperglycaemia occurred. In contrast of these findings the serum glucose content in non-pregnant rats exposed to Ni-concentrations of 0.8 and 1.6 mg/m3 were content in non-pregnant rats exposed to Ni-concentrations of 0.8 and 1.6 mg/m3 were significantly reduced. After NiO inhalation (1.6 and 3.2 mg/m3), exposure signs of a marked macrocytosis occurred in pregnant and non-pregnant rats. The oral application of NiCl2 in drinking water in male rats induced a significant decrease of urea in serum and a significant increase of urea in urine. The activity of alkaline phosphatase in serum was inhibited in male rats exposed to 0.4 and 0.8 mg/m3 NiO-aerosols. No significant difference in serum protein pattern and no 'nickeloplasmin' was detected by serumelectrophoresis and tandem-crossed immunoelectrophoresis after oral inhalation exposure in male rats. Fetuses of exposed dams showed in the groups receiving 1.6 and 3.2 mg/m3 significantly reduced body weights.

A selective effect of Ni2+ on wave initiation in bull sperm flagella.
Lindemann C. B., Fentie I. and Rikmenspoel R.
The J Cell Biol. 1980;87(2 Pt 1):420-6.

Bull sperm that are extracted with 0.1% Triton X-100 and restored to motility with Mg2+-ATP lose coordination and stop swimming in the presence of 0.5 mM NiSO4. Although spontaneous coordination of flagellar waves is lost after exposure to Ni2+, other functions of the flagellum remain intact. The capacity for wave propagation along the flagellum is maintained together with the capacity for microtubular sliding. Wave motility can be restored to Ni2+-inhibited sperm by inducing a permanent bend onto the

flagellum by micromanipulation. In the absence of such intervention, the loss of wave coordination is complete and irreversible. Ni2+-inhibited demembranated cells that are kept active by maintaining a bend in the flagellum exhibit a normal beat frequency. Both intact and demembranated sperm can retain spontaneous wave production at considerably slower rates of motion than Ni2+-inhibited cells. Short segments from the distal tip of the flagellum contain only the 9 + 2 microtubular axoneme. These short segments are able to propagate imposed bends even in the presence of Ni2+. In addition to wave propagation Ni2+-treated sperm can be shown to exhibit a normal sliding tubule phenomenon by direct assay. Although Ni2+-treated cells have a functional sliding tubule mechanism, and consequently the axoneme can propagate bends, it appears that these retained functions are not sufficient to cause spontaneous bend initiation. Our findings show that bend initiation is inhibited by Ni2+, and therefore is an independent process separate from the sliding tubule mechanism responsible for wave propagation.

D. Titles only (abstracts not available)

[Effects of oxidative stress on nickel sulfate induced testis impairment in mice].
Sun Y. B., Wang X. X., Song Y. C., Wang J. L. and Zhu Y. Z.
Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi (Chinese journal of industrial hygiene and occupational diseases). 2006;24(7):425-6.

Spermatotoxic effects of nickel in mice.
Pandey R. and Srivastava S. P.
B Env Cont Toxicol. 2000;64(2):161-7.

Distribution and kinetics of injected nickel in the pregnant rat.
Mas A., Peligero M. J., Arola L. and Alemany M.
Clin Exp Pharmacol Physiol. 1986;13:91-96.

Immunological studies in mice following in utero exposure to nicl2.
Smialowicz R. J., Rogers R. R., Riddle M. M., Rowe D. G. and Luebke R. W.
Toxicology. 1986;38:293-303.

Teratogenic effects of nickel acetate on chick embryogenesis.
King D. W. and Chen D. C.
Sheng Su K'O Hsueh. 1984;23:44-51.

Effects of Metal on Sea Urchin development: A Rapid Bioassay
Lee, H. H. and Xu, C. H.
Mar Pollut Bull 1984; 15: 18-21

Placental transfer and body distribution of nickel chloride in pregnant mice.
Lu C. C., Matsumoto N. and Iijima S.
Toxicol Appl Pharmacol. 1981;59:409-13.

Nickel deprivation in rats:nickel-iron interactions.
Nielsen F. H., Zimmerman T. J., Collings M. E. and Myron D. R.
J Nutr. 1979;109:1623-32.

Effects of aluminium and nickel on survival and reproduction in polychaetous annelids.
Petrich S. M. and Reish D. J.
Bull Environ Contam Toxicol. 1979;23(4-5):698-702.

Whole-body autoradiography of 63Ni in mice throughout gestation.
Olsen I. and Jonsen J.
Toxicology. 1979;12:165-72.

Effect of the administration of trace amounts of metals to pregnant mice upon the behavior and learning of their offspring.
Tsujii H. and Hoshishima K.
Shinshu Daigaku Nogakubu Kiyo. 1979;16:13-28.

Nickel and strontium distribution in some mouse tissues:passage through placenta and mammary glands.
Jacobsen N., Alfheim I. and Jonsen J.
Res Commun Chem Pathol Pharmacol. 1978;20:571-84.

Embryotoxicity and fetal toxicity of nickel in rats.
Sunderman F. W., Jr., Shen S. K., Mitchell J. M., Allpass P. R. and Damjanov I.
Toxicol Appl Pharmacol. 1978;43(2):381-90.

Effect of metal ions on collagen synthesis in embryonic rat calvaria.
Srivastava R., Lefebvre N. and Onkelinx C.
Proc Eur Soc Toxicol. 1976;17:191-97.

[Testicular changes due to long-term administration of nickel sulphate in rats].
Waltschewa W., Slatewa M. and Michailow I.
Exper Pathol. 1972;6(3):116-20.

Toxic effects of trace elements on the reproduction of mice and rats.
Schroeder H. A. and Mitchener M.
Arch Environ Health. 1971;23:102-06.

Effect of nickel supplementation on production and composition of milk.
O'Dell G. D., Miller W. J., King W. A., Ellers J. C. and Jurecek H.
J. Dairy Sci. 1970;53(11):1545-8.

Effect of intrauterine copper and other metals on implantation in rats and hamsters.
Chang C. C., Tatum H. J. and Kincl F. A.
Fertil Steril. 1970;21:274-78.

APPENDIX: PERFLUOROOCTANOIC ACID (PFOA)

Perfluorooctanoic acid (PFOA, also known as C8; CAS # 335-67-1) is a synthetic chemical that is used to make most fluoropolymers, which are substances with many manufacturing and industrial applications. For example, fluoropolymers are used to make non-stick surfaces for cookware, stain repellent treatments, waterproof and breathable membranes for clothing, and make materials fire-resistant. PFOA can also be produced by the breakdown of some fluorinated telomers, which are used to make product surfaces resistant to soil, stains, grease, and water.

This document presents a compilation of abstracts of articles on the developmental and reproductive toxicity of PFOA identified during our epidemiological screen and subsequent toxicological evaluation. OEHHA originally screened PFOA in 2007 but there was not sufficient human data available for PFOA to pass the screen at that time. We applied an epidemiologic data screen on PFOA in 2015. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. PFOA passed the epidemiologic screen. We also conducted a preliminary toxicological evaluation searching for relevant studies, including animal studies.

OEHHA used the information in this document to select PFOA for presentation to the Developmental and Reproductive Toxicant Identification Committee as a possible candidate for Committee consideration. The abstracts compiled below are from epidemiologic and animal toxicity studies reporting on developmental and reproductive sequelae related to exposure to PFOA, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species).

Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Thirty-four epidemiologic studies of PFOA reporting increased risk of adverse developmental or reproductive outcomes were identified, twenty of which were analytical studies of adequate quality. Nine studies reported increased risk of adverse developmental or reproductive outcomes that were not statistically significant. Thirty-seven epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified, as well as

four studies which had unclear findings. A further fifty-eight related articles were identified.

In addition, the following animal toxicity studies were identified.

- Twenty animal studies of PFOA reporting reproductive or developmental toxicity were identified. Two animal studies reporting no reproductive or developmental toxicity were identified. Thirty-six related articles and one study with no abstract were also identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

i. Studies that were statistically significant

[*]Prenatal Exposure to Perfluoroalkyl Acids and Serum Testosterone Concentrations at 15 Years of Age in Female ALSPAC Study Participants.
Maisonet M., Calafat A. M., Marcus M., Jaakkola J. J. and Lashen H.
Environ Health Perspect. 2015 Jun 2. [Epub ahead of print].

BACKGROUND: Exposure to perfluorooctane sulfonic acid (PFOS) or to perfluorooctanoic acid (PFOA) increases mouse and human PPARα subtype activity which influences lipid metabolism. Because cholesterol is the substrate from which testosterone is synthesized exposure to these substances has the potential to alter testosterone concentrations. OBJECTIVES: Explore associations of total testosterone and sex hormone-binding globulin (SHBG) concentrations at age 15 with prenatal exposures to PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) in females. METHODS: Prenatal concentrations of the perfluoroalkyl acids (PFAAs) were measured in serum collected from pregnant mothers at enrollment (1991-1992) in the Avon Longitudinal Study of Parents and Children (ALSPAC). The median gestational age when the maternal blood sample was obtained was 16 weeks (interquartile range: 11- 28 weeks). Total testosterone and SHBG concentrations were measured in serum obtained from their daughters at 15 years of age. Associations between prenatal PFAAs concentrations and reproductive outcomes were estimated using linear regression models (n=72). RESULTS: Adjusted total testosterone concentrations were on average 0.18 nmol/L (95% CI: 0.01, 0.35) higher in daughters with prenatal PFOS in the upper concentration tertile compared to daughters with prenatal PFOS in the lower tertile. Adjusted total testosterone concentrations were also higher in daughters with prenatal concentrations of PFOA (β = 0.24; 95% CI: 0.05, 0.43) and PFHxS (β = 0.18; 95% CI: 0.00, 0.35) in the upper tertile compared to daughters with concentrations in the lower tertile. We did not find evidence of associations between PFNA and total testosterone or between any of the PFAAs and SHBG. CONCLUSIONS: Our findings were based on a small study sample

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

and should be interpreted with caution. However, they suggest that prenatal exposure to some PFAAs may alter testosterone concentrations in females.

[*]Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females.

Maisonet M., Nayha S., Lawlor D. A. and Marcus M.
Environ Int. 2015;82:49-60.

BACKGROUND: In some cross-sectional epidemiologic studies the shape of the association between serum concentrations of perfluoroalkyl acids (PFAAs) and lipids suggests departures from linearity. OBJECTIVES: We used statistical approaches allowing for non-linearity to determine associations of prenatal exposures of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) with lipid concentrations. METHODS: PFAAs were measured in serum from pregnant women collected in 1991-1992 at enrollment in the Avon Longitudinal Study of Parents and Children and lipids in serum from their daughters at ages 7 (n=111) and 15 (n=88). The associations of PFAAs with lipids were first explored by cubic splines, followed by piecewise linear regressions by tertiles to obtain regression coefficients (β) and their 95% confidence limits (95% CL) (in mg/dL per 1ng/mL). RESULTS: At age 7, total cholesterol was positively associated with prenatal PFOA concentrations in the lower tertile (β=15.01; 95% CL=2.34, 27.69) but not with PFOA concentrations in the middle (β=-3.63; 95% CL=-17.43, 10.16) and upper (β=-1.58; 95% CL=-4.58, 1.42) tertiles. At age 15, a similar pattern was noted as well. Positive associations between LDL-C and prenatal PFOA concentration in the lower tertile were observed in daughters at ages 7 (β=14.91; 95% CL=3.53, 28.12) and 15 (β=13.93; 95% CL=0.60, 27.26). LDL-C was not associated with PFOA concentrations in the middle or upper tertile at any age. Neither HDL-C nor triglycerides was associated with prenatal PFOA exposure. Non-linear patterns of association of total cholesterol and LDL-C with prenatal PFOS were less consistently noted. CONCLUSION: Exposure to low levels of PFOA during prenatal development may alter lipid metabolism later in life. Given the small sample size further replication of the association in large independent cohorts is important.

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[*]Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years--a prospective study.

Hoyer B. B., Ramlau-Hansen C. H., Obel C., Pedersen H. S., Hernik A., Ogniev V., Jonsson B. A., Lindh C. H., Rylander L., Rignell-Hydbom A., Bonde J. P. and Toft G. Environ Health. 2015;14:2.

BACKGROUND: In animal studies, perfluorinated alkyl substances affect growth and neuro-behavioural outcomes. Human epidemiological studies are sparse. The aim was to investigate the association between pregnancy serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and offspring behaviour and motor development at 5-9 years of age. METHODS: Maternal sera from the INUENDO cohort (2002-2004) comprising 1,106 mother-child pairs from Greenland, Kharkiv (Ukraine) and Warsaw (Poland) were analysed for PFOS and PFOA, using liquid-chromatography-tandem-mass-spectrometry. Exposures were grouped into country specific as well as pooled tertiles as well as being used as continuous variables for statistical analyses. Child motor development and behaviour at follow-up (2010-2012) were measured by the Developmental Coordination Disorder Questionnaire 2007 (DCDQ) and Strength and Difficulties Questionnaire (SDQ), respectively. Exposure-outcome associations were analysed by multiple logistic and linear regression analyses. RESULTS: In the pooled analysis, odds ratio (OR) (95% confidence interval (CI)) for hyperactivity was 3.1 (1.3, 7.2) comparing children prenatally exposed to the highest PFOA tertile with those exposed to the lowest PFOA tertile. Comparing children in the highest PFOS tertile with those in the lowest PFOS tertile showed elevated but statistically non-significant OR of hyperactivity (OR (95% CI) 1.7 (0.9, 3.2)). In Greenland, elevated PFOS was associated with higher SDQ-total scores indicating more behavioural problems (β (95% CI)=1.0 (0.1, 2.0)) and elevated PFOA was associated with higher hyperactivity sub-scale scores indicating more hyperactive behaviour (β (95% CI)=0.5 (0.1, 0.9)). Prenatal PFOS and PFOA exposures were not associated with motor difficulties. CONCLUSIONS: Prenatal exposure to PFOS and PFOA may have a small to moderate effect on children's neuro-behavioural development, specifically in terms of hyperactive behaviour. The associations were strongest in Greenland where exposure contrast is largest.

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***A prospective study of prepregnancy serum concentrations of perfluorochemicals and the risk of gestational diabetes.**
Zhang C., Sundaram R., Maisog J., Calafat A. M., Barr D. B. and Buck Louis G. M.
Fertil Steril. 2015;103(1):184-9.

OBJECTIVE: To examine preconception serum concentrations of perfluorooctanoic acid (PFOA) and six other PFCs in relation to gestational diabetes (GDM) risk. DESIGN: Prospective cohort with longitudinal follow-up. SETTING: Not applicable. PATIENT(S): Among 501 women recruited upon discontinuing contraception for the purpose of becoming pregnant, 258 (51%) became pregnant and were eligible for the study, of which 28 (11%) reported having physician-diagnosed GDM during follow-up. INTERVENTION(S): None. MAIN OUTCOME MEASURE(S): The odds ratios (ORs) and 95% confidence intervals (CIs) of GDM associated with each standard deviation (SD) increment of preconception serum PFOA concentration (ng/mL, log-transformed) and six other PFCs were estimated with the use of logistic regression after adjusting for age, prepregnancy body mass index, smoking, and parity conditional on gravidity. RESULT(S): Preconception geometric mean (95% CI) PFOA concentrations (in ng/mL) were higher for women with than without GDM (3.94 [3.15-4.93] vs. 3.07 [2.83-3.12], respectively). Each SD increment in PFOA was associated with a 1.87-fold increased GDM risk (adjusted OR 1.86 [95% CI 1.14-3.02]). A slightly increased risk associated with each SD increment for the six other PFCs was observed as well (all ORs >1.0, range 1.06-1.27), although the associations were not statistically significant. CONCLUSION(S): Our findings suggested that higher environmentally relevant concentrations of PFOA were significantly associated with an increased risk of GDM. If corroborated, these findings may be suggestive of a possible environmental etiology for GDM.

***Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior.**
Donauer S., Chen A., Xu Y., Calafat A. M., Sjodin A. and Yolton K.
J Pediatr. 2015;166: 736-42.

OBJECTIVE: To assess the impact of prenatal exposure to polybrominated diphenyl ethers (PBDEs) and polyfluoroalkyl chemicals (PFCs) on early infant neurobehavior.

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STUDY DESIGN: In a cohort of 349 mother/infant pairs, we measured maternal serum concentrations during pregnancy of PBDEs, including BDE-47 and other related congeners, as well as 2 common PFCs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid. When the infants were 5 weeks of age, we measured their neurobehavior by using the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS).
RESULTS: Neither PBDE nor PFC exposures during gestation were associated with the 11 individual NNNS outcomes included in our study; however, when we used latent profile analysis to categorize infants into neurobehavioral profiles based on performance on the NNNS (social/easygoing, high arousal/difficult, or hypotonic), a 10-fold increase in prenatal PFOA concentrations significantly increased the odds of being categorized as hypotonic compared with social/easygoing (aOR 3.79; 95% CI 1.1-12.8).
CONCLUSIONS: Infants of mothers with greater serum concentrations of PFOA during pregnancy were more likely to be categorized as hypotonic. No association between PBDE concentrations and hypotonia was found. Additional studies should further investigate possible associations of prenatal PFC exposure and muscle tone in infants and children.

***Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study.**
de Cock M., de Boer M. R., Lamoree M., Legler J. and van de Bor M.
Environ Health. 2014;13(106).

BACKGROUND: Endocrine disrupting chemicals (EDCs) present in the environment may disrupt thyroid hormones, which in early life are essential for brain development. Observational studies regarding this topic are still limited, however as the presence of chemicals in the environment is ubiquitous, further research is warranted. The objective of the current study was to assess the association between exposure markers of various EDCs and thyroxine (T4) levels in newborns in a mother-child cohort in the Netherlands. METHODS: Exposure to dichlorodiphenyldichloroethylene (DDE), three di-2-ethylhexyl phthalate (DEHP) metabolites, hexachlorobenzene (HCB), polychlorinated biphenyl (PCB)-153, perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA) was determined in cord plasma or breast milk, and information on T4 levels in heel prick blood spots was obtained through the neonatal screening programme in the Netherlands. Linear regression models were composed to

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determine associations between each of the compounds and T4, which were stratified for gender and adjusted for a priori defined covariates. RESULTS: Mean T4 level was 86.9 nmol/L (n = 83). Girls in the highest quartile of DDE and PFOA exposure showed an increased T4 level compared to the lowest quartile with both crude and fully adjusted models (DDE > 107.50 ng/L, +24.8 nmol/L, 95% CI 0.79, 48.75; PFOA > 1200 ng/L, +38.6 nmol/L, 95% CI 13.34, 63.83). In boys a lower T4 level was seen in the second quartile of exposure for both PFOS and PFOA, however after fully adjusting the models these associations were attenuated. No effects were observed for the other compounds. CONCLUSION: DDE and perfluorinated alkyl acids may be associated with T4 in a sex-specific manner. These results should however be interpreted with caution, due to the relatively small study population. More research is warranted, as studies on the role of environmental contaminants in this area are still limited.

Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study.
Webster G. M., Venners S. A., Mattman A. and Martin J. W.
Environ Res. 2014;133:338-47.

BACKGROUND: Associations between perfluoroalkyl acids (PFASs) and human thyroid hormone levels remain unclear, especially during early pregnancy when small changes in maternal thyroid hormones can affect fetal brain development. OBJECTIVES: To examine associations between maternal serum PFAS levels and maternal thyroid hormone levels in the early 2nd trimester of pregnancy. METHODS: Participants were euthyroid pregnant women (n=152) enrolled in the Chemicals, Health and Pregnancy (CHirP) study based in Vancouver, Canada. Associations between maternal serum PFASs, including perfluorohexanesulfonate (PFHxS), perfluorononanoate (PFNA), perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and repeated measures of maternal thyroid hormones, including free thyroxine (fT4), total thyroxine (TT4) and thyroid stimulating home (TSH) were examined using mixed effects linear models. Associations were considered in all women, then separately in women with high (≥ 9 IU/mL) vs normal (<9 IU/mL) levels of thyroid peroxidase antibody (TPOAb), a marker of autoimmune hypothyroidism (Hashimoto's disease). RESULTS: Median PFAS concentrations (ng/mL) in maternal sera were 1.0 (PFHxS), 0.6 (PFNA), 1.7 (PFOA) and 4.8 (PFOS). PFASs were not associated with fT4, TT4 or TSH among women with normal TPOAb. However, among the 9% of women with high TPOAb (n=14), interquartile range (IQR) increases of PFASs were associated with a 46-69% increase in maternal TSH (95% CIs ranging from 8% to 123%) (PFNA, PFOA and PFOS only), and with a 3% to 7% decrease in maternal fT4

(95% CIs ranging from -18% to 5%) (all 4 PFASs). PFNA was also associated with higher maternal TSH in the whole sample. CONCLUSIONS: PFASs were positively associated with TSH, and weakly negatively associated with fT4 in the subset of pregnant women with high TPOAb, which occurs in 6-10% of pregnancies. PFASs may exacerbate the already high TSH and low fT4 levels in these women during early pregnancy, which is a critical time of thyroid hormone-mediated fetal brain development. The clinical significance of these findings is not clear. We propose a "multiple hit hypothesis" to explain these findings; this hypothesis deserves evaluation in larger, more representative study samples.

***Perfluorooctanoate exposure and major birth defects.**
Stein C. R., Savitz D. A., Elston B., Thorpe P. G. and Gilboa S. M.
Reprod Toxicol. 2014;47:15-20.

Perfluorooctanoate (PFOA) is detectable in umbilical cord blood and amniotic fluid. Some toxicological findings suggest that perfluoroalkyl substances may be teratogenic. Using data from the C8 Health Project, a 2005-2006 survey in a Mid-Ohio Valley community exposed to PFOA through contaminated drinking water, we examined the association between estimated prenatal PFOA concentration and maternally reported birth defects (n=325) among 10,262 live singleton or multiple births from 1990 to 2006. Logistic regression models accounted for siblings using generalized estimating equations. There was generally no association between estimated PFOA concentration and birth defects, with the possible exception of brain defects, where the odds ratio adjusted for year of conception was 2.6 (95% confidence interval 1.3-5.1) for an increase in estimated PFOA exposure from the 25th to 75th percentile. This estimate, however, was based on 13 cases and may represent a chance finding. Further investigation of this potential association may be warranted.

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Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study.

Vagi S. J., Azziz-Baumgartner E., Sjodin A., Calafat A. M., Dumesic D., Gonzalez L., Kato K., Silva M. J., Ye X. and Azziz R.

BMC Endocr Disord. 2014;14:86.

BACKGROUND: Polycystic Ovary Syndrome (PCOS) is an endocrine-metabolic disorder that affects approximately 6-10% of women of child-bearing age. Although preliminary studies suggest that certain pollutants may act as endocrine disruptors in animals, little is known about their potential association with PCOS. The objective of this case-control pilot study is to determine whether women with PCOS have higher concentrations of specific environmental contaminants compared to women who have not developed PCOS. METHODS: Fifty-two PCOS case-patients (diagnosed using the National Institutes of Health 1990 definition) and 50 controls were recruited in 2007-2008, from an urban academic medical center in Los Angeles, CA. Brominated diphenyl ethers, polychlorinated biphenyls (PCBs), organochlorine pesticides, and perfluorinated compounds (PFCs) were measured in serum, and phthalates metabolites and bisphenol A (BPA) in urine. RESULTS: PCOS case-patients had significantly higher geometric mean (GM) serum concentrations of two PFCs: perfluorooctanoate (PFOA) (GMcases = 4.1 mug/L, GMcontrols = 2.3 mug/L; p = 0.001) and perfluorooctane sulfonate (PFOS) (GMcases = 8.2 mug/L, GMcontrols = 4.9 mug/L; p = 0.01), and lower urinary concentrations of monobenzyl phthalate (mBzP) (GMcases = 7.5 mug/g creatinine, GMcontrols = 11.7 mug/g creatinine; p = 0.02). Logistic regression, controlling for body mass index, age and race, identified an increased likelihood of PCOS in subjects with higher serum concentrations of PFOA and PFOS (adjusted-ORs = 5.8-6.9, $p < 0.05$), and with lower urine concentrations of mBzP and mono-n-butyl phthalate (mBP) (aORs = 0.14-0.25, $p < 0.05$). CONCLUSIONS: Our data suggest that PCOS case-patients may differ from controls in their environmental contaminant profile. PCOS subjects had higher serum concentrations of two PFCs, PFOA and PFOS, and lower urine concentrations of mBP and mBzP. Future studies are needed to confirm these preliminary findings and determine if these chemicals or their precursors may have a role in the pathogenesis of PCOS.

Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas.

La Rocca C., Tait S., Guerranti C., Busani L., Ciardo F., Bergamasco B., Stecca L., Perra G., Mancini F. R., Marci R., Bordi G., Caserta D., Focardi S., Moscarini M. and Mantovani A.
Int J Environ Res Public Health. 2014;11(10):10146-64.

Within the PREVIENI project, infertile and fertile women were enrolled from metropolitan, urban and rural Italian areas. Blood/serum levels of several endocrine disrupters (EDs) (perfluorooctane sulfonate, PFOS; perfluorooctanoic acid, PFOA; di-2-ethylhexyl-phthalate, DEHP; mono-(2-ethylhexyl)-phthalate, MEHP; bisphenol A, BPA) were evaluated concurrently with nuclear receptors (NRs) gene expression levels (ERa, ERb, AR, AhR, PPARg, PXR) in peripheral blood mononuclear cells (PBMCs). Infertile women from the metropolitan area displayed significantly higher levels of: BPA compared to fertile women (14.9 vs. 0.5 ng/mL serum); BPA and MEHP compared to infertile women from urban and rural areas; enhanced expression levels of NRs, except PPARg. Infertile women from urban and rural areas had PFOA levels significantly higher than those from metropolitan areas. Our study indicates the relevance of the living environment when investigating the exposure to EDs and the modulation of the NR panel in PBMC as a suitable biomarker of the effect, to assess the EDs impact on reproductive health.

[*]Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children.
Liew Z., Ritz B., Bonefeld-Jírgensen E. C., Henriksen T. B., Nohr E. A., Bech B. H., Fei C., Bossi R., von Ehrenstein O. S., Streja E., Uldall P. and Olsen J.
Am J Epidemiol. 2014;180:574-81.

Perfluoroalkyl substances (PFASs) are persistent pollutants and endocrine disruptors that may affect fetal brain development. We investigated whether prenatal exposure to PFASs increases the risk of congenital cerebral palsy (CP). The source population for this study includes 83,389 liveborn singletons and mothers enrolled in the Danish National Birth Cohort during 1996-2002. We identified 156 CP cases by linking the cohort to the Danish National Cerebral Palsy Register, and we randomly selected 550 controls using a case-cohort design. We measured 16 PFASs in maternal plasma collected in early or midpregnancy, and 6 PFASs were quantifiable in more than 90%

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of the samples. We found a higher risk of CP in boys with higher maternal PFAS levels; per 1-unit (natural-log ng/mL) increase, the risk ratios were 1.7 (95% confidence interval: 1.0, 2.8) for perfluorooctane sulfonate and 2.1 (95% confidence interval: 1.2, 3.6) for perfluorooctanoic acid. We also observed a dose-response pattern of CP risk in boys per quartile of maternal level of perfluorooctane sulfonate and perfluorooctanoic acid (P for trend < 0.01). PFASs were associated with both unilateral and bilateral spastic CP subphenotypes. No association between PFASs and CP was found in girls. Prenatal exposures to PFASs may increase the risk of CP in boys, but the finding is novel and replication is needed.

Perfluoroalkyl substances in human milk: a first survey in Italy.
Barbarossa A., Masetti R., Gazzotti T., Zama D., Astolfi A., Veyrand B., Pession A. and Pagliuca G.
Environ Int. 2013;51:27-30.

Due to their widespread diffusion, perfluoroalkyl substances (PFASs) have been frequently found in the environment and in several animal species. It has been demonstrated that they can easily reach also humans, mainly through diet. Being lactation a major route of elimination of these contaminants, their occurrence in human milk is of particular interest, especially considering that it generally represents the unique food source for newborns. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the two most important compounds of this family, have been frequently found in human milk at variable concentrations, but still limited data are available. The present study, the first conducted in Italy capable to detect these pollutants at ultra-trace levels by UPLC-MS/MS, confirmed the role of lactation as a relevant source of exposure for breastfed children. The measured concentrations ranged between 15 and 288 ng/L for PFOS and between 24 and 241 ng/L for PFOA. Moreover, mean concentrations and frequencies of both analytes resulted higher in milk samples provided by primiparous women, suggesting that the risk of intake might be higher for first-borns. Finally, comparing these results with previous data, PFOS gradual decrease over time since year 2000 was confirmed.

Association between thyroid profile and perfluoroalkyl acids: data from NHANES 2007-2008.
Jain R. B.
Environ Res. 2013;126:51-9.

The effect of six perfluoroalkyl acids (PFAAs), namely, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDE),

perfluorohexane sulfonic acid (PFHxS), 2-(N-methyl-perfluorooctane sulfonamide) acetic acid (MPAH), and perfluorononanoic acid (PFNA) on the levels of six thyroid function variables, namely, thyroid stimulating hormone (TSH), free and total thyroxine (FT4, TT4), free and total triiodothyronine (FT3, TT3), and thyroglobulin (TGN) was evaluated. Data from National Health and Nutrition Examination Survey for the years 2007-2008 were used for this evaluation. TSH levels increased with increase in levels of PFOA ($p<0.01$). There were no statistically significant associations between the levels of FT3, and FT4 with the levels of any of the six PFAAs. Levels of TT3 were found to increase with the levels of PFOA ($p=0.01$) and TT4 levels were found to increase with increase in PFHxS levels ($p<0.01$). Males had statistically significantly higher levels of FT3 than females and females had statistically significantly higher levels of TT4 than males. As compared to non-Hispanics whites and Hispanics, non-Hispanic blacks had lower levels of TSH, FT3, TT3, and TT4 but Hispanics had the lowest levels of TGN. Age was negatively associated with FT3 and TT3 but positively associated with FT4 and TT4. Non-smokers had higher levels of TSH and TT4 than smokers and smokers had higher levels of FT3 and TGN than non-smokers. Iodine deficiency was associated with increased levels of TSH, TT3, TT4, and TGN.

***Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010.**
Darrow L. A., Stein C. R. and Steenland K.
Environ Health Perspect. 2013;121(10):1207-13.

BACKGROUND: Previous research suggests perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) may be associated with adverse pregnancy outcomes. OBJECTIVE: We conducted a population-based study of PFOA and PFOS and birth outcomes from 2005 through 2010 in a Mid-Ohio Valley community exposed to high levels of PFOA through drinking-water contamination. METHODS: Women provided serum for PFOA and PFOS measurement in 2005-2006 and reported reproductive histories in subsequent follow-up interviews. Reported singleton live births among 1,330 women after 1 January 2005 were linked to birth records (n = 1,630) to identify the outcomes of preterm birth (< 37 weeks gestation), pregnancy-induced hypertension, low birth weight (< 2,500 g), and birth weight (grams) among full-term infants. RESULTS: We observed little or no evidence of association between maternal serum PFOA or PFOS and preterm birth (n = 158) or low birth weight (n = 88). Serum PFOA and PFOS were both positively associated with pregnancy-induced

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hypertension (n = 106), with adjusted odds ratios (ORs) per log unit increase in PFOA and PFOS of 1.27 (95% CI: 1.05, 1.55) and 1.47 (95% CI: 1.06, 2.04), respectively, but associations did not increase monotonically when categorized by quintiles. Results of subanalyses restricted to pregnancies conceived after blood collection were consistent with the main analyses. There was suggestion of a modest negative association between PFOS and birth weight in full-term infants (-29 g per log unit increase; 95% CI: -66, 7), which became stronger when restricted to births conceived after the blood sample collection (-49 g per log unit increase; 95% CI: -90, -8). CONCLUSION: Results provide some evidence of positive associations between measured serum perfluorinated compounds and pregnancy-induced hypertension and a negative association between PFOS and birth weight among full-term infants.

Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea.
Lee Y. J., Kim M. K., Bae J. and Yang J. H.
Chemosphere. 2013;90(5):1603-9.

This study analyzed the concentrations of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) in maternal and umbilical cord sera at delivery from the general population in Korea. Seventy samples were analyzed with ion-pairing and LC/MS/MS. PFOS, PFOA and PFHxS were detected in both maternal and umbilical cord sera. There was a high correlation of PFC concentrations between maternal and cord serum samples, implying transplacental transport. Ranking of transplacental transfer efficiency was PFOA>PFHxS>PFOS. Student's t-tests revealed that concentrations of maternal PFOA were related with decreases in birth weight, birth length and ponderal index, suggesting a possible impact on fetal growth. With multiple logistic regression models, maternal PFOS concentration showed a significant inverse association with ponderal index (OR=0.22; 95% CI, 0.05-0.90). Umbilical cord PFHxS concentration showed a significant inverse association with birth weight (OR=0.26; 95% CI, 0.08-0.85) or a marginally significant inverse association with birth length (OR=0.33; 95% CI, 0.09-1.17). This is the first report demonstrating an inverse association of birth outcomes with PFHxS exposure. Concentrations of maternal PFOA were decreased with parity, implying that delivery is one of the major routes for PFOA elimination in women. This study demonstrated prenatal exposure of PFCs through placental transfer which could result in possible developmental effects in the population sampled. Our results may provide data basis to conduct a larger scale investigation into developmental effects of PFCs in the future and contribute to understanding levels of PFC contaminations from a variety of populations in the globe.

[*]Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction.

Kristensen S. L., Ramlau-Hansen C. H., Ernst E., Olsen S. F., Bonde J. P., Vested A., Halldorsson T. I., Becher G., Haug L. S. and Toft G.

Hum Reprod. 2013;28:3337-48.

STUDY QUESTION: Does prenatal exposure to perfluoroalkyl substances (PFASs) have long-term effects on female reproductive function?.

SUMMARY ANSWER: Our results suggest an association between in utero exposure to perfluorooctanoic acid (PFOA) and delay in age of menarche.

WHAT IS KNOWN ALREADY: Previous cross-sectional studies have reported possible effects of PFASs on female reproduction including reduced fecundity, delayed puberty and accelerated age at menopause. Only limited data exist from follow-up studies on long-term implications of prenatal exposure to PFASs.

STUDY DESIGN, SIZE, DURATION: In this study we used data from a Danish population-based cohort established in 1988-1989. Of 1212 eligible pregnant women, 965 participated. Follow-up was initiated in 2008 on the female offspring at approximately 20 years of age. Three hundred and sixty seven (84%) daughters answered a questionnaire and 267 (61%) daughters furthermore attended clinical examinations which were conducted in 2008-2009.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The final study population consisted of 343 daughters of which 254 had attended the clinical examinations and 89 had answered the questionnaire only. Levels of PFASs in maternal serum from pregnancy week 30 were used as a measure of prenatal exposure and related to age of menarche, menstrual cycle length, levels of reproductive hormones and follicle number of the daughters. Data were divided into three groups according to tertiles of maternal concentrations of PFASs (low, medium, high).

MAIN RESULTS AND THE ROLE OF CHANCE: In adjusted regression analyses, daughters exposed to higher levels of PFOA in utero had a 5.3 (95% confidence interval: 1.3; 9.3) months later age of menarche compared with the reference group of lower PFOA. Crude (P = 0.05) and adjusted (P = 0.01) trend tests also indicated a relationship between higher prenatal PFOA exposure and delay of menarche.

LIMITATIONS, REASONS FOR CAUTION: We did not measure the exact amount of PFASs to which the daughters had been exposed prenatally. Instead we used PFAS concentrations in maternal serum as surrogates. However, PFASs are efficiently

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transferred to the fetus via placenta. Information on age of menarche was collected retrospectively but the time interval for recall in our study was relatively short (2-10 years). The remaining outcome measures depended on participation in clinical examination which reduced the number of observations leading to limited statistical power and risk of selection bias.
WIDER IMPLICATIONS OF THE FINDINGS: Since PFASs can be detected in humans all over the world, effects of prenatal exposure on female reproductive function later in life may have wide health implications.
STUDY FUNDING/COMPETING INTEREST(S): The study was supported by the Danish Council for Independent Research (271-05-0296, 09-065631), the Danish Ministry of Interior and Health (0-302-02-18/5), the Danish Council for Strategic Research (09-067124 (Centre for Fetal Programming), 09-063072, 2101-06-0005), the Novo Nordisk Foundation, the Aarhus University Research Foundation, the Frimodt-Heineke Foundation, the Foundation of Maria Dorthea and Holger From, the Beckett-Foundation, the Research Grant of Organon and the Foundation of Lily Benthine Lund. There are no competing interests.
TRIAL REGISTRATION NUMBER: Not applicable.

[*]Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men.
Vested A., Ramlau-Hansen C. H., Olsen S. F., Bonde J. P., Kristensen S. L., Halldorsson T. I., Becher G., Haug L. S., Ernst E. H. and Toft G.
Environ Health Perspect. 2013;121:453-8.

BACKGROUND: Perfluorinated alkyl acids (PFAAs), persistent chemicals with unique water-, dirt-, and oil-repellent properties, are suspected of having endocrine-disrupting activity. The PFAA compounds perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are found globally in humans; because they readily cross the placental barrier, in utero exposure may be a cause for concern.
OBJECTIVES: We investigated whether in utero exposure to PFOA and PFOS affects semen quality, testicular volume, and reproductive hormone levels.
METHODS: We recruited 169 male offspring (19-21 years of age) from a pregnancy cohort established in Aarhus, Denmark, in 1988-1989, corresponding to 37.6% of the eligible sons. Each man provided a semen sample and a blood sample. Semen samples were analyzed for sperm concentration, total sperm count, motility, and morphology, and blood samples were used to measure reproductive hormones. As a

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proxy for in utero exposure, PFOA and PFOS were measured in maternal blood samples from pregnancy week 30.
RESULTS: Multivariable linear regression analysis suggested that in utero exposure to PFOA was associated with lower adjusted sperm concentration (ptrend = 0.01) and total sperm count (ptrend = 0.001) and with higher adjusted levels of luteinizing hormone (ptrend = 0.03) and follicle-stimulating hormone (ptrend = 0.01). PFOS did not appear to be associated with any of the outcomes assessed, before or after adjustment.
CONCLUSIONS: The results suggest that in utero exposure to PFOA may affect adult human male semen quality and reproductive hormone levels.

[*]Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood.
Granum B., Haug L. S., Namork E., Stílevik S. B., Thomsen C., Aaberge I. S., van Loveren H., Lívik M. and Nygaard U. C.
J Immunotoxicol. 2013;10:373-9.

Perfluoroalkyl substances (PFAS) are suggested to have immunosuppressive effects; exposure in utero and in the first years of life is of special concern as fetuses and small children are highly vulnerable to toxicant exposure. The objective of this study was to investigate the effect of pre-natal exposure to PFAS on responses to pediatric vaccines and immune-related health outcomes in children up to 3 years of age. In the prospective birth-cohort BraMat, a sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa), pregnant women from Oslo and Akershus, Norway, were recruited during 2007-2008. Three annual questionnaire-based follow-ups were performed. Blood samples were collected from the mothers at the time of delivery and from the children at the age of 3 years. As a measure of pre-natal exposure to PFAS, the concentrations of perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were determined in maternal blood from 99 BraMat participants. Main outcome measures were anti-vaccine antibody levels, common infectious diseases and allergy- and asthma-related health outcomes in the children up to the age of 3 years. There was an inverse association between the level of anti-rubella antibodies in the children's serum at age 3 years and the concentrations of the four PFAS. Furthermore, there was a positive association between the maternal concentrations of PFOA and PFNA and the number of episodes of common cold for the children, and between PFOA and PFHxS

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and the number of episodes of gastroenteritis. No associations were found between maternal PFAS concentrations and the allergy- and asthma-related health outcomes investigated. The results indicate that pre-natal exposure to PFAS may be associated with immunosuppression in early childhood.

Perfluorochemicals and endometriosis: the ENDO study.
Louis G. M., Peterson C. M., Chen Z., Hediger M. L., Croughan M. S., Sundaram R., Stanford J. B., Fujimoto V. Y., Varner M. W., Giudice L. C., Kennedy A., Sun L., Wu Q. and Kannan K.
Epidemiology. 2012;23(6):799-805.

BACKGROUND: Environmental chemicals may be associated with endometriosis. No published research has focused on the possible role of perfluorochemicals (PFCs) despite their widespread presence in human tissues. METHODS: We formulated two samples. The first was an operative sample comprising 495 women aged 18-44 years scheduled for laparoscopy/laparotomy at one of 14 participating clinical sites in the Salt Lake City or San Francisco area, 2007-2009. The second was a population-based sample comprising 131 women matched to the operative sample on age and residence within a 50-mile radius of participating clinics. Interviews and anthropometric assessments were conducted at enrollment, along with blood collection for the analysis of nine PFCs, which were quantified using liquid chromatography-tandem mass spectrometry. Endometriosis was defined based on surgical visualization (in the operative sample) or magnetic resonance imaging (in the population sample). Using logistic regression, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) for each PFC (log-transformed), adjusting for age and body mass index, and then parity. RESULTS: Serum perfluorooctanoic acid (PFOA; OR = 1.89 [95% CI = 1.17-3.06]) and perfluorononanoic acid (2.20 [1.02-4.75]) were associated with endometriosis in the operative sample; findings were moderately attenuated with parity adjustment (1.62 [0.99-2.66] and 1.99 [0.91-4.33], respectively). Perfluorooctane sulfonic acid (1.86 [1.05-3.30]) and PFOA (2.58 [1.18-5.64]) increased the odds for moderate/severe endometriosis, although the odds were similarly attenuated with parity adjustment (OR = 1.50 and 1.86, respectively). CONCLUSIONS: Select PFCs were associated with an endometriosis diagnosis. These associations await corroboration.

[*]Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls.

Maisonet M., Terrell M. L., McGeehin M. A., Christensen K. Y., Holmes A., Calafat A. M. and Marcus M.

Environ Health Perspect. 2012;120(10):1432-7.

BACKGROUND: Prenatal exposures to polyfluoroalkyl compounds (PFCs) may be associated with adverse changes in fetal and postnatal growth. OBJECTIVE: We explored associations of prenatal serum concentrations of perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexane sulfonate (PFHxS) with fetal and postnatal growth in girls. METHODS: We studied a sample of 447 singleton girls and their mothers participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). Data on weight and length were obtained at birth and at 2, 9, and 20 months. Serum samples were obtained in 1991-1992, from mothers during pregnancy. We explored associations between prenatal PFC concentrations and weight at birth as well as longitudinal changes in weight-for-age SD scores between birth and 20 months. RESULTS: PFOS (median, 19.6 ng/mL), PFOA (median, 3.7 ng/mL), and PFHxS (median, 1.6 ng/mL) were detected in 100% of samples. On average, girls born to mothers with prenatal concentrations of PFOS in the upper tertile weighed 140 g less [95% confidence interval (CI): -238, -42] at birth than girls born to mothers with concentrations in the lower tertile in adjusted models. Similar patterns were seen for PFOA (-133 g; 95% CI: -237, -30) and PFHxS (-108 g; 95% CI: -206, -10). At 20 months, however, girls born to mothers with prenatal concentrations of PFOS in the upper tertile weighed 580 g more (95% CI: 301, 858) when compared with those in the lower tertile. No differences in weight were found for PFOA and PFHxS. CONCLUSIONS: Girls with higher prenatal exposure to each of the PFCs examined were smaller at birth than those with lower exposure. In addition, those with higher exposure to PFOS were larger at 20 months.

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[*]Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes.

Wu K., Xu X., Peng L., Liu J., Guo Y. and Huo X.
Environ Int. 2012;48:1-8.

OBJECTIVE: Perfluorooctanoic acid (PFOA) has applications in numerous industrial and consumer products. The widespread prevalence of PFOA in humans demonstrated in recent studies has drawn considerable interest from the public. We aimed to evaluate the exposure of mothers to PFOA and the potential hazards to neonates in a primitive electronic waste recycling area, Guiyu, China, and a control area, Chaonan, China. METHODS: Our investigation included analyses of maternal serum samples, health effect examinations, and other relevant factors. Questionnaires were administered and maternal serum samples were collected for 167 pregnant women. Solid phase extraction method was used for all analytical sample preparation, and analyses were completed using high performance liquid chromatography tandem mass spectrometry method. RESULTS: The PFOA concentration was higher in maternal serum samples from Guiyu than in samples from Chaonan (median 16.95, range 5.5-58.5 ng mL(-1); vs. 8.7, range 4.4-30.0 ng mL(-1); P<0.001). Residence in Guiyu, involvement in e-waste recycling, husband's involvement in e-waste and use of the family residence as workshop were significant factors contributing to PFOA exposure. Maternal PFOA concentrations were significantly different between normal births and adverse birth outcomes including premature delivery, term low birth weight, and stillbirths. After adjusting for potential confounders, PFOA was negatively associated with gestational age [per lg-unit: β=-15.99 days, 95% confidence interval (CI), -27.72 to -4.25], birth weight (per lg-unit: β=-267.3g, 95% CI, -573.27 to -37.18), birth length (per lg-unit: β=-1.91 cm, 95% CI, -3.31 to -0.52), and Apgar scores (per lg-unit: β=-1.37, 95% CI, -2.42 to -0.32), but not associated with ponderal index. CONCLUSIONS: Mothers from Guiyu were exposed to higher levels of PFOA than those from control areas. Prenatal exposure to PFOA was associated with decreased neonatal physical development and adverse birth outcomes.

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[*]Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study.

Halldorsson T. I., Rytter D., Haug L. S., Bech B. H., Danielsen I., Becher G., Henriksen T. B. and Olsen S. F.
Environ Health Perspect. 2012;120(5):668-73.

BACKGROUND: Perfluoroalkyl acids are persistent compounds used in various industrial -applications. Of these compounds, perfluorooctanoate (PFOA) is currently detected in humans worldwide. A recent study on low-dose developmental exposure to PFOA in mice reported increased weight and elevated biomarkers of adiposity in postpubertal female offspring. OBJECTIVE: We examined whether the findings of increased weight in postpubertal female mice could be replicated in humans. METHODS: A prospective cohort of 665 Danish pregnant women was recruited in 1988-1989 with offspring follow-up at 20 years. PFOA was measured in serum from gestational week 30. Offspring body mass index (BMI) and waist circumference were recorded at follow-up (n = 665), and biomarkers of adiposity were quantified in a subset (n = 422) of participants. RESULTS: After adjusting for covariates, including maternal pre-pregnancy BMI, smoking, education, and birth weight, in utero exposure to PFOA was positively associated with anthropometry at 20 years in female but not male offspring. Adjusted relative risks comparing the highest with lowest quartile (median: 5.8 vs. 2.3 ng/mL) of maternal PFOA concentration were 3.1 [95% confidence interval (CI): 1.4, 6.9] for overweight or obese (BMI ≥ 25 kg/m2) and 3.0 (95% CI: 1.3, 6.8) for waist circumference > 88 cm among female offspring. This corresponded to estimated increases of 1.6 kg/m2 (95% CI: 0.6, 2.6) and 4.3 cm (95% CI: 1.4, 7.3) in average BMI and waist circumference, respectively. In addition, maternal PFOA concentrations were positively associated with serum insulin and leptin levels and inversely associated with adiponectin levels in female offspring. Similar associations were observed for males, although point estimates were less precise because of fewer observations. Maternal perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA), and perfluorononanoate (PFNA) concentrations were not independently associated with offspring anthropometry at 20 years. CONCLUSIONS: Our findings on the effects of low-dose developmental exposures to PFOA are in line with experimental results suggesting obesogenic effects in female offspring at 20 years of age.

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Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants.
Okada E., Sasaki S., Saijo Y., Washino N., Miyashita C., Kobayashi S., Konishi K., Ito Y. M., Ito R., Nakata A., Iwasaki Y., Saito K., Nakazawa H. and Kishi R.
Environ Res. 2012;112:118-25.

BACKGROUND: Recent studies have shown effects of prenatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on infants in the general environmental levels. Laboratory animal studies have shown that exposure to PFOS and PFOA is associated with immunotoxic effects. OBJECTIVES: To investigate the relationship between maternal PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. Cord blood immunoglobulin (Ig) E levels were also evaluated. METHODS: We conducted a prospective cohort study of pregnant women from 2002 to 2005 in Sapporo, Japan. Maternal PFOS and PFOA levels were measured in relation to cord blood IgE concentrations (n=231) and infant allergies and infectious diseases (n=343). Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at 18 months of age. Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured. RESULTS: Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis media in the 18 month-old infants (adjusted for confounders). CONCLUSIONS: Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases at age in 18 months.

Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project.
Knox S. S., Jackson T., Frisbee S. J., Javins B. and Ducatman A. M.
J Toxicol Sci. 2011;36(4):403-10.

Perfluorocarbons from common household products such as food containers, stain-resistant protection for clothing, furniture and carpets, paints, and fire-fighting foams are found in soil, water, plants, animal and human serum worldwide. Previous research has shown a significant association between these chemicals and thyroid disease in women. The present data from the C8 Health Project assessed thyroid function in a cross-sectional analysis of 52,296 adults with a year or more of exposure to

perfluorooctanoate (PFOA) from drinking water. Outcomes were: thyroxine, T3 uptake, and thyroid stimulating hormone (TSH). Analyses were stratified by gender and age group (< 20 - < 50 years and > 50). Both PFOA and perfluorooctane sulfonate (PFOS) were associated with significant elevations in serum thyroxine and a significant reduction in T3 uptake in all participants. There were also significant gender/PFOS interactions for T3 uptake and thyroxine, as well as gender/PFOA interactions for T3 uptake. Results provide evidence for disruption of thyroid function related to these common chemicals and possible mechanisms are discussed.

Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant.
Lopez-Espinosa M. J., Fletcher T., Armstrong B., Genser B., Dhatariya K., Mondal D., Ducatman A. and Leonardi G.
Environ Sci Technol. 2011;45(19):8160-6.

Animal studies suggest that perfluorocarbons (PFCs) may alter sexual maturation. Relationships of human PFC exposure with puberty are not clear. We conducted a cross-sectional study to investigate whether perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were associated with indicators of sexual maturation in a 2005-2006 survey of residents with PFOA water contamination from the Mid-Ohio Valley. Participants were 3076 boys and 2931 girls aged 8-18 years. They were classified as having reached puberty based on either hormone levels (total >50 ng/dL and free >5 pg/mL testosterone in boys and estradiol >20 pg/mL in girls) or onset of menarche. We estimated the odds of having reached puberty classified by these criteria and the fitted median age of reaching puberty in relation to serum PFOA and PFOS concentrations measured when puberty status was assigned. For boys, there was a relationship of reduced odds of reached puberty (raised testosterone) with increasing PFOS (delay of 190 days between the highest and lowest quartile). For girls, higher concentrations of PFOA or PFOS were associated with reduced odds of postmenarche (130 and 138 days of delay, respectively). In conclusion, our study showed a later age of puberty in this population correlated with PFC concentrations.

Implications of early menopause in women exposed to perfluorocarbons.
Knox S. S., Jackson T., Javins B., Frisbee S. J., Shankar A. and Ducatman A. M.
J Clin Endocrinol Metab. 2011;96(6):1747-53.

CONTEXT: Perfluorocarbons (PFC) are man-made chemicals used in numerous household products. They have a long half-life in humans and complex animal toxicity, and accumulating evidence points toward associations with multiple human health

endpoints. OBJECTIVE: Our objective was to investigate whether PFC are associated with endocrine disruption in women. DESIGN: Cross-sectional analyses were made between quintiles of serum PFC, serum estradiol, and menopause onset. SETTING: The C8 Health Project, with cohort of 69,030 adults and children, was conducted due to PFC contamination of drinking water from six water districts in two states. PARTICIPANTS: Participants included 25,957 women aged 18-65 yr. MAIN OUTCOME MEASURES: Serum estradiol levels and onset of menopause were assessed. The survey was the result of a class action suit, and survey designers (an independent corporation) had no a priori hypotheses. All hypotheses have been formulated by other investigators after data collection. RESULTS: After excluding women who reported hysterectomy and adjusting for age within the group, smoking, alcohol consumption, body mass index, and exercise, the odds of having experienced menopause were significantly higher in the highest quintile relative to the lowest quintile of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) in the perimenopausal [PFOS odds = 1.4, confidence interval (CI) = 1.1-1.8; PFOA odds =1.4, CI = 1.1-1.8] and menopausal age groups (PFOS odds = 2.1, CI=1.6-2.8; PFOA odds = 1.7, CI = 1.3-2.3). After appropriate exclusions and adjustment for covariates, there was a significant inverse association between PFOS and estradiol in perimenopausal (β = -3.65; $P < 0.0001$) and menopausal age groups (β = -0.83; $P = 0.007$) but not between PFOA and estradiol. CONCLUSIONS: These data suggest that PFC are associated with endocrine disruption in women and that further research on mechanisms is warranted.

***The effect of prenatal perfluorinated chemicals exposures on pediatric atopy.**
Wang I. J., Hsieh W. S., Chen C. Y., Fletcher T., Lien G. W., Chiang H. L., Chiang C. F., Wu T. N. and Chen P. C.
Environ Res. 2011;111:785-91.

BACKGROUND: The role of perfluorinated compounds (PFCs) in the immune system and allergic diseases is not well-known. This study examined the effects of pre-natal exposure to PFCs on immunoglobulin E (IgE) levels and atopic dermatitis (AD). METHODS: In Taiwan Birth Panel cohort study, newborns with cord blood and peri-natal factors (i.e. birth body weight, weeks of gestation, and type of delivery) gathered at birth were evaluated. At the age of 2 years, information on the development of AD, environmental exposures, and serum total IgE were collected. The AD and non-AD children were compared for the concentration of cord blood serum PFCs measured by

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Ultra-performance liquid chromatography/triple-quadrupole mass (UPLC-MS/MS). Correlations among cord blood IgE, serum total IgE at 2 years of age, and cord blood PFC levels were made.
RESULTS: Of 244 children who completed the follow-up and specimen collections, 43 (17.6%) developed AD. Concentrations of cord blood serum perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were median (range) 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 (0.035-0.420)ng/mL, respectively. PFOA and PFOS levels positively correlated with cord blood IgE levels (per ln-unit: β =0.134 KU/l, p=0.047 for PFOA; β =0.161 KU/l, p=0.017 for PFOS). Analyses stratified by gender revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels only in boys (per ln-unit: β =0.206 KU/l, p=0.025 for PFOA; β =0.175 KU/l, p=0.053 for PFOS). When dividing cord blood serum PFCs into quartiles in the fully adjusted models, AD had no significant association with PFOS.
CONCLUSIONS: Pre-natal PFOA and PFOS exposures positively correlated with cord blood IgE levels.

***Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding.**
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Scand J Work Environ Health.
2010;36(5):413-21.

OBJECTIVE: Perfluorooctanoate (PFOA) has been associated with impaired lactation in mice. We examined whether maternal perfluorooctanesulfonate (PFOS) and PFOA concentrations correlated with duration of breastfeeding among women. METHODS: We randomly selected 1400 pregnant women from the Danish national birth cohort (1996-2002) and measured PFOS and PFOA concentrations in early pregnancy by using high performance liquid chromatography/tandem mass spectrometry. Self-reported data on the duration of any and exclusive breastfeeding were collected twice during telephone interviews around 6 and 18 months after the birth of the child.
RESULTS: The duration of breastfeeding decreased with increasing concentrations of pregnancy PFOS and PFOA among multiparous women, for whom the adjusted odds ratios (OR) for weaning before 6 months of age were 1.20 (95% CI 1.06-1.37) per 10 ng/ml increase in PFOS concentrations and 1.23 (95% CI 1.13-1.33) per 1 ng/ml increase in PFOA concentrations. No consistent association was found for primiparous

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women. CONCLUSIONS: These findings suggest that PFOA and PFOS may reduce the ability to lactate, but could equally reflect reverse causation since no association was seen in primiparous women.

***Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy.**

Andersen C. S., Fei C., Gamborg M., Nohr E. A., Sorensen T. I. and Olsen J.
Am J Epidemiol. 2010;172:1230-7.

Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are persistent chemicals that may affect growth early in life. The authors estimated the associations between maternal plasma levels of PFOS and PFOA and infants' weight, length, and body mass index development during the first year of life. Fourteen hundred women were randomly selected from the Danish National Birth Cohort among those who provided blood samples early in pregnancy and gave birth to liveborn singletons between 1996 and 2002. Weight and length information at 5 and 12 months of age was available for 1,010 children. Multiple linear regression models were used for analyses, and maternal PFOS and PFOA concentrations (ng/mL) were inversely related to children's weight in the first year of life: adjusted regression coefficients: 0.8 g (95% confidence interval(CI): 4.2, 2.6) at 5 months and 5.8 g (95% CI:10.4, 1.2) at 12 months for perfluorooctanesulfonate(PFOS); 9.4 g (95% CI: 28.6, 9.9) at 5 months and 19.0 g (95% CI: 44.9, 6.8) at 12 months for perfluorooctanoate(PFOA) [corrected]. A similar pattern was observed for body mass index measurements, and no associations with length were found. After sex stratification, the inverse associations with weight and body mass index were more pronounced in boys, and no clear association was seen for girls.

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Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water.

Nolan L. A., Nolan J. M., Shofer F. S., Rodway N. V. and Emmett E. A.
Reprod Toxicol. 2010;29(2):147-55.

BACKGROUND: We have previously examined the associations between perfluorooctanoic acid (PFOA) exposure, birth weight and gestational age in individuals exposed to PFOA-contaminated residential drinking water from the Little Hocking Water Association (LHWA). In this investigation, we expand the scope of our analysis to examine the associations between PFOA, congenital anomalies, labor and delivery complications and maternal risk factors. OBJECTIVES: To compare the likelihood of congenital anomalies, labor and delivery complications and maternal risk factors in neonates and their mothers residing in zip codes with public water service provided completely, partially or not at all by the LHWA. METHODS: Logistic regression analyses were performed on singleton neonatal birth outcome data supplied by the Ohio Department of Health to examine the associations between LHWA water service category and the outcomes of interest. When possible, models were adjusted for maternal age, preterm birth, neonatal sex, race, maternal education, alcohol use, tobacco use and diabetic status. RESULTS: Increased PFOA exposure, as assessed by water service category, was not associated with an overall increase in the likelihood of congenital anomalies or any specific diagnosis (adjusted OR: 1.4, 95% CI: 0.34-3.3). The overall likelihood of labor and delivery complications was significantly lower among mothers with water service provided by the LHWA, as compared to mothers not serviced by the LHWA (adjusted OR: 0.65, 95% CI: 0.46-0.92). A significant increase in the likelihood of anemia (crude OR: 11, 95% CI: 1.8-64) and dysfunctional labor (crude OR: 5.3, 95% CI: 1.2-24) was noted for mothers residing within zip codes serviced by the LHWA, but the number of reported cases was very small. CONCLUSION: At the levels measured in the LHWA, we conclude that PFOA is not associated with increased risk of congenital anomalies, most labor and delivery complications and maternal risk factors. Additional research is required to assess the observed associations between PFOA, anemia and dysfunctional labor.

Maternal levels of perfluorinated chemicals and subfecundity.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Hum Reprod. 2009;24(5):1200-5.

BACKGROUND: Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are ubiquitous man-made compounds that are possible hormonal disruptors. We examined whether exposure to these compounds may decrease fecundity in humans. METHODS: Plasma levels of PFOS and PFOA were measured at weeks 4-14 of pregnancy among 1240 women from the Danish National Birth Cohort recruited from 1996 to 2002. For this pregnancy, women reported time to pregnancy (TTP) in five categories (<1, 1-2, 3-5, 6-12 and >12 months). Infertility was defined as having a TTP of >12 months or received infertility treatment to establish this pregnancy. RESULTS: Longer TTP was associated with higher maternal levels of PFOA and PFOS ($P < 0.001$). Compared with women in the lowest exposure quartile, the adjusted odds of infertility increased by 70-134 and 60-154% among women in the higher three quartiles of PFOS and PFOA, respectively. Fecundity odds ratios (FORs) were also estimated using Cox discrete-time models. The adjusted FORs were virtually identical for women in the three highest exposure groups of PFOS (FOR = 0.70, 0.67 and 0.74, respectively) compared with the lowest quartile. A linear-like trend was observed for PFOA (FOR = 0.72, 0.73 and 0.60 for three highest quartiles versus lowest quartile). When all quartiles were included in a likelihood ratio test, the trends were significant for PFOS and PFOA ($P = 0.002$ and $P < 0.001$, respectively). CONCLUSIONS: These findings suggest that PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity; such exposure levels are common in developed countries.

[*]Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort.
Fei C., McLaughlin J. K., Tarone R. E. and Olsen J.
Am J Epidemiol. 2008;168(1):66-72.

Perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) are widespread persistent organic pollutants that have been associated with reduced birth weight at doses expected in many pregnant populations. The authors randomly selected 1,400 pregnant women and their newborns from the Danish National Birth Cohort (1996-2002) to investigate whether these compounds reduce organ growth. PFOS and PFOA

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

were measured in maternal blood samples taken early in pregnancy. Placental weight, birth length, and head and abdominal circumferences were measured shortly after birth by trained midwives or nurses. Maternal PFOA levels in early pregnancy were associated with smaller abdominal circumference and birth length. For each ng/ml increase in PFOA, birth length decreased by 0.069 cm (95% confidence interval: 0.024, 0.113) and abdominal circumference decreased by 0.059 cm (95% confidence interval: 0.012, 0.106). An inverse association was also observed between PFOA and placental weight and head circumference, and a positive association was observed with newborn ponderal index, but none of these associations was statistically significant. Maternal PFOS levels were not associated with any of the five fetal growth indicators. These findings suggest that fetal exposure to PFOA but not PFOS during organ development may affect the growth of organs and the skeleton.

***Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort.**

Fei C., McLaughlin J. K., Tarone R. E. and Olsen J.
Environ Health Perspect. 2007;115(11):1677-82.

BACKGROUND: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are man-made, persistent organic pollutants widely spread throughout the environment and human populations. They have been found to interfere with fetal growth in some animal models, but whether a similar effect is seen in humans is uncertain. OBJECTIVES: We investigated the association between plasma levels of PFOS and PFOA in pregnant women and their infants' birth weight and length of gestation. METHODS: We randomly selected 1,400 women and their infants from the Danish National Birth Cohort among those who completed all four computer-assisted telephone interviews, provided the first blood samples between gestational weeks 4 and 14, and who gave birth to a single live-born child without congenital malformation. PFOS and PFOA were measured by high performance liquid chromatography-tandem mass spectrometer. RESULTS: PFOS and PFOA levels in maternal plasma were on average 35.3 and 5.6 ng/mL, respectively. Only PFOA levels were inversely associated with birth weight (adjusted β = -10.63 g; 95% confidence interval, -20.79 to -0.47 g). Neither maternal PFOS nor PFOA levels were consistently associated with the risk for preterm birth or low birth weight. We observed no adverse effects for maternal PFOS or PFOA levels on small for gestational age. CONCLUSION: Our nationwide cohort data suggest an inverse association between maternal plasma

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

PFOA levels and birth weight. Because of widespread exposure to these chemicals, our findings may be of potential public health concern.

Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden.
Karrman A., Ericson I., van Bavel B., Darnerud P. O., Aune M., Glynn A., Lignell S. and Lindstrom G.
Environ Health Perspect. 2007;115(2):226-30.

BACKGROUND: Only limited data exist on lactation as an exposure source of persistent perfluorinated chemicals (PFCs) for children. OBJECTIVES: We studied occurrence and levels of PFCs in human milk in relation to maternal serum together with the temporal trend in milk levels between 1996 and 2004 in Sweden. Matched, individual human milk and serum samples from 12 primiparous women in Sweden were analyzed together with composite milk samples (25-90 women/year) from 1996 to 2004. RESULTS: Eight PFCs were detected in the serum samples, and five of them were also above the detection limits in the milk samples. Perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. Perfluorooctanesulfonamide (PFOSA), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected less frequently. DISCUSSION: The total PFC concentration in maternal serum was 32 ng/mL, and the corresponding milk concentration was 0.34 ng/mL. The PFOS milk level was on average 1% of the corresponding serum level. There was a strong association between increasing serum concentration and increasing milk concentration for PFOS (r(2) = 0.7) and PFHxS (r(2) = 0.8). PFOS and PFHxS levels in composite milk samples were relatively unchanged between 1996 and 2004, with a total variation of 20 and 32% coefficient of variation, respectively. CONCLUSION: The calculated total amount of PFCs transferred by lactation to a breast-fed infant in this study was approximately 200 ng/day. Lactation is a considerable source of exposure for infants, and reference concentrations for hazard assessments are needed.

ii. Studies that were not statistically significant

Anthropometry in 5- to 9-Year-Old Greenlandic and Ukrainian Children in Relation to Prenatal Exposure to Perfluorinated Alkyl Substances.
Hoyer B. B., Ramlau-Hansen C. H., Vrijheid M., Valvi D., Pedersen H. S., Zviezdai V., Jonsson B. A., Lindh C. H., Bonde J. P. and Toft G.
Environ Health Perspect. 2015 123(8):841-6.

BACKGROUND: In some animal studies, perfluorinated alkyl substances are suggested to induce weight gain. Human epidemiological studies investigating these associations are sparse. OBJECTIVE: To examine pregnancy serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and the prevalence of offspring overweight (> 1 standard deviation) and waist-to-height ratio (WHtR) > 0.5 at five to nine years of age. METHODS: Sera from 1,022 pregnant women enrolled in the INUENDO cohort (2002-2004) from Greenland and Kharkiv (Ukraine) were analysed for PFOA and PFOS using liquid chromatography-tandem-mass-spectrometry. Relative risks (RR) of being overweight and having WHtR > 0.5 in relation to continuous and categorised (tertiles) PFOA and PFOS were calculated at follow-up (2010-2012) using generalised linear models. RESULTS: Pooled PFOA median (range) was 1.3 (0.2-5.1) and PFOS median (range) was 10.8 (0.8-73.0) ng/mL. For each natural logarithm-unit (ln-unit) increase of pregnancy PFOA, the adjusted RR of offspring overweight was 1.11 (95% confidence interval (CI): 0.82, 1.53) in Greenlandic children. In Ukrainian children, the adjusted RR of offspring overweight was 1.02 (95% CI: 0.72, 1.44) for each ln-unit increase of pregnancy PFOA. Prenatal exposure to PFOS was not associated with overweight in country-specific or pooled analysis. The adjusted RR of having WHtR > 0.5 for each ln-unit increase of prenatal exposure to PFOA was 1.30 (95% CI: 0.97, 1.74) in the pooled analysis. For one ln-unit increase of prenatal exposure to PFOS, the adjusted RR of having a WHtR > 0.5 was 1.38 (95% CI: 1.05, 1.82) in the pooled analysis. CONCLUSIONS: The results indicate that prenatal PFOA and PFOS exposure may be associated with child waist-to-height ratio > 0.5. Prenatal PFOA and PFOS exposure were not associated with overweight.

Prenatal Phthalate, Perfluoroalkyl Acid, and Organochlorine Exposures and Term Birth Weight in Three Birth Cohorts: Multi-Pollutant Models Based on Elastic Net Regression.

Lenters V., Portengen L., Rignell-Hydbom A., Jonsson B. A., Lindh C. H., Piersma A. H., Toft G., Bonde J. P., Heederik D., Rylander L. and Vermeulen R.
Environ Health Perspect. 2015.

BACKGROUND: Some legacy and emerging environmental contaminants are suspected risk factors for intrauterine growth restriction. However, the evidence is equivocal, in part due to difficulties in disentangling the effects of mixtures. OBJECTIVES: We assessed associations between multiple correlated biomarkers of environmental exposure and birth weight. METHODS: We evaluated a cohort of 1250 term (≥37 weeks' gestation) singleton infants, born to 513 mothers from Greenland, 180 from Poland, and 557 from Ukraine, who were recruited during antenatal care visits in 20022004. Secondary metabolites of diethylhexyl and diisononyl phthalates (DEHP, DiNP), eight perfluoroalkyl acids, and organochlorines (PCB-153 and p,p'-DDE) were quantifiable in 72100% of maternal serum samples. We assessed associations between exposures and term birth weight, adjusting for co-exposures and covariates, including pre-pregnancy body mass index. To identify independent associations, we applied the elastic net penalty to linear regression models. RESULTS: Two phthalate metabolites (MEHHP, MOiNP), perfluorooctanoate (PFOA), and p,p'-DDE were most consistently predictive of term birth weight based on elastic net penalty regression. In an adjusted, unpenalized regression model of the four exposures, 2-SD increases in natural log-transformed MEHHP, PFOA, and p,p'-DDE were associated with lower birth weight: -87 g (95% CI: -137, -340 per 1.70 ng/mL), -43 g (95% CI: -108, 23; per 1.18 ng/mL), and -135 g (95% CI: -192, -78 per 1.82 ng/g lipid), respectively; while MOiNP was associated with higher birth weight (46 g; 95% CI: -5, 97 per 2.22 ng/mL). CONCLUSIONS: This study suggests that several of the environmental contaminants, belonging to three chemical classes, may be independently associated with impaired fetal growth. These results warrant follow-up in other cohorts.

Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study.
Lyngso J., Ramlau-Hansen C. H., Hoyer B. B., Stovring H., Bonde J. P., Jonsson B. A., Lindh C. H., Pedersen H. S., Ludwicki J. K., Zviezdai V. and Toft G.
Hum Reprod. 2014;29(2):359-67.

STUDY QUESTION: Does perfluorooctane sulfonate (PFOS) and perfluorooctanate (PFOA) exposure disrupt the menstrual cyclicity? SUMMARY ANSWER: The female reproductive system may be sensitive to PFOA exposure, with longer menstrual cycle length at higher exposure. WHAT IS KNOWN ALREADY: PFOS and PFOA are persistent man-made chemicals. Experimental animal studies suggest they are reproductive toxicants but epidemiological findings are inconsistent. STUDY DESIGN, SIZE, DURATION: A cross-sectional study including 1623 pregnant women from the INUENDO cohort enrolled during antenatal care visits between June 2002 and May 2004 in Greenland, Poland and Ukraine. PARTICIPANTS/MATERIALS, SETTING, METHODS: Information on menstrual cycle characteristics was obtained by questionnaires together with a blood sample from each pregnant woman. Serum concentrations of PFOS and PFOA were measured by liquid chromatography tandem mass spectrometry. Multiple imputations were performed to account for missing data. The association between PFOS/PFOA and menstrual cycle length (short cycle: ≤24 days, long cycle: ≥32 days) and irregularities (≥7 days in difference between cycles) was analyzed using logistic regression with tertiles of exposure. Estimates are given as adjusted odds ratios (ORs) with 95% confidence intervals (CIs). MAIN RESULTS AND THE ROLE OF CHANCE: Higher exposure levels of PFOA were associated with longer menstrual cycles in pooled estimates of all three countries. Compared with women in the lowest exposure tertile, the adjusted OR of long cycles was 1.8 (95% CI: 1.0; 3.3) among women in the highest tertile of PFOA exposure. No significant associations were observed between PFOS exposure and menstrual cycle characteristics. However, we observed a tendency toward more irregular cycles with higher exposure to PFOS [OR 1.7 (95% CI: 0.8; 3.5)]. The overall response rate was 45.3% with considerable variation between countries (91.3% in Greenland, 69.1% in Poland and 26.3% in Ukraine). LIMITATIONS, REASONS FOR CAUTION: Possible limitations in our study include varying participation rates across countries; a selected study group overrepresenting the most fertile part of the population; retrospective information on menstrual cycle characteristics; the determination of cut-points for all three outcome variables; and lacking information on some determinants of menstrual cycle characteristics, such as stress, physical activity, chronic diseases and gynecological disorders, thus confounding cannot be excluded. WIDER IMPLICATIONS OF THE FINDINGS: The generalizability of the study results is

restricted to fertile women who manage to conceive and women who do not use oral contraceptives when getting pregnant or within 2 months before getting pregnant. To our knowledge only one previous epidemiological study has addressed the possible association between perfluorinated chemical exposure and menstrual disturbances. Though pointing toward different disturbances in cyclicity, both studies suggest that exposure to PFOA may affect the female reproductive function. This study contributes to the limited knowledge on effects of exposure to PFOA and PFOS on female reproductive function and suggests that the female reproductive system may be affected by environmental exposure to PFOA. STUDY FUNDING/COMPETING INTEREST(S): Supported by a scholarship from Aarhus University Research Foundation. The collection of questionnaire data and blood samples was part of the INUENDO project supported by The European Commission (Contract no. QLK4-CT-2001-00 202), www.inuendo.dk. The Ukrainian part of the study was possible by a grant from INTAS (project 012 2205). Determination of PFOA and PFOS in serum was part of the CLEAR study (www.inuendo.dk/clear) supported by the European Commission's 7th Framework Program (FP7-ENV-2008-1-226217). No conflict of interest declared.

Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants.

Arbuckle T. E., Kubwabo C., Walker M., Davis K., Lalonde K., Kosarac I., Wen S. W. and Arnold D. L.

Int J Hyg Environ Health. 2013;216(2):184-94.

Perfluoroalkyl acids (PFAAs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants representing two classes of environmental contaminants of toxicological concern, especially for infants. Canadian biomonitoring data on these chemicals are limited. The objectives of this study were to measure PFAAs and PBDEs in umbilical cord blood from approximately 100 hospital deliveries in Ottawa (Ontario, Canada) and examine associations with characteristics of the mother and infant. Geometric means were 1.469 ng/mL for perfluorooctanoate (PFOA) (95% confidence interval of 1.292-1.671 ng/mL), 4.443 ng/mL for perfluorooctane sulfonate (PFOS) (95% CI of 3.735-5.285 ng/mL), 0.359 ng/mL for perfluorononanoic acid (PFNA) (95% CI of 0.318-0.404 ng/mL), and 0.579 ng/mL for perfluorohexanesulfonate (PFHxS) (95% CI of 0.473-0.709 ng/mL). The final multiple regression models indicated that lower gravida, term gestational age, smoking during pregnancy and vaginal delivery were significantly associated with higher levels of PFOS. Similarly, a vaginal delivery was significantly associated with higher PFOA, while weak associations were found with lower gravida and birth weight less than 2500 g.

Furthermore, higher PFNA concentrations were significantly associated with older mothers, and vaginal delivery, while weakly associated with term gestational age. Elevated PFHxS concentrations were significantly associated with smoking during pregnancy and lower gravida. Similar to reports from other countries, the preponderant PBDE congener measured in the cord blood was PBDE-47. Questions remain on why various studies have reported conflicting results on the association between PFAAs and birth weight.

Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study.
Whitworth K. W., Haug L. S., Baird D. D., Becher G., Hoppin J. A., Skjaerven R., Thomsen C., Eggesbo M., Travlos G., Wilson R., Cupul-Uicab L. A., Brantsaeter A. L. and Longnecker M. P.
Am J Epidemiol. 2012;175(12):1209-16.

Perfluorooctane sulfonate and perfluorooctanoic acid are perfluorinated compounds (PFCs) widely distributed in the environment. Previous studies of PFCs and birth weight are equivocal. The authors examined this association in the Norwegian Mother and Child Cohort Study (MoBa), using data from 901 women enrolled from 2003 to 2004 and selected for a prior case-based study of PFCs and subfecundity. Maternal plasma samples were obtained around 17 weeks of gestation. Outcomes included birth weight z scores, preterm birth, small for gestational age, and large for gestational age. The adjusted birth weight z scores were slightly lower among infants born to mothers in the highest quartiles of PFCs compared with infants born to mothers in the lowest quartiles: for perfluorooctane sulfonate, β = -0.18 (95% confidence interval: -0.41, 0.05) and, for perfluorooctanoic acid, β = -0.21 (95% confidence interval: -0.45, 0.04). No clear evidence of an association with small for gestational age or large for gestational age was observed. Perfluorooctane sulfonate and perfluorooctanoic acid were each associated with decreased adjusted odds of preterm birth, although the cell counts were small. Whether some of the associations suggested by these findings may be due to a noncausal pharmacokinetic mechanism remains unclear.

Perfluorinated compounds and subfecundity in pregnant women.
Whitworth K. W., Haug L. S., Baird D. D., Becher G., Hoppin J. A., Skjaerven R., Thomsen C., Eggesbo M., Travlos G., Wilson R. and Longnecker M. P.
Epidemiology. 2012;23:257-63.

BACKGROUND: Perfluorinated compounds are ubiquitous pollutants; epidemiologic data suggest they may be associated with adverse health outcomes, including

subfecundity. We examined subfecundity in relation to 2 perfluorinated compounds-perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).
METHODS: This case-control analysis included 910 women enrolled in the Norwegian Mother and Child Cohort Study in 2003 and 2004. Around gestational week 17, women reported their time to pregnancy and provided blood samples. Cases consisted of 416 women with a time to pregnancy greater than 12 months, considered subfecund. Plasma concentrations of perfluorinated compounds were analyzed using liquid chromatography-mass spectrometry. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each pollutant quartile using logistic regression. Estimates were further stratified by parity.
RESULTS: The median plasma concentration of PFOS was 13.0 ng/mL (interquartile range [IQR] = 10.3-16.6 ng/mL) and of PFOA was 2.2 ng/mL (IQR = 1.7-3.0 ng/mL). The relative odds of subfecundity among parous women was 2.1 (95% CI = 1.2-3.8) for the highest PFOS quartile and 2.1 (1.0-4.0) for the highest PFOA quartile. Among nulliparous women, the respective relative odds were 0.7 (0.4-1.3) and 0.5 (0.2-1.2).
CONCLUSION: Previous studies suggest that the body burden of perfluorinated compounds decreases during pregnancy and lactation through transfer to the fetus and to breast milk. Afterward, the body burden may increase again. Among parous women, increased body burden may be due to a long interpregnancy interval rather than the cause of a long time to pregnancy. Therefore, data from nulliparous women may be more informative regarding toxic effects of perfluorinated compounds. Our results among nulliparous women did not support an association with subfecundity.

Thyroid function and perfluoroalkyl acids in children living near a chemical plant.
Lopez-Espinosa M. J., Mondal D., Armstrong B., Bloom M. S. and Fletcher T.
Environ Health Perspect. 2012;120(7):1036-41.

BACKGROUND: Animal studies suggest that some perfluoroalkyl acids (PFAAs), including perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), and perfluorononanoic acid (PFNA) may impair thyroid function. Epidemiological findings, mostly related to adults, are inconsistent. OBJECTIVES: We investigated whether concentrations of PFAAs were associated with thyroid function among 10,725 children (1-17 years of age) living near a Teflon manufacturing facility in the Mid-Ohio Valley (USA). METHODS: Serum levels of thyroid-stimulating hormone (TSH), total thyroxine (TT4), and PFAAs were measured during 2005-2006, and information on diagnosed thyroid disease was collected by questionnaire. Modeled in utero PFOA concentrations were based on historical information on PFOA releases, environmental distribution, pharmacokinetic modeling, and residential histories. We performed multivariate

regression analyses. RESULTS: Median concentrations of modeled in utero PFOA and measured serum PFOA, PFOS, and PFNA were 12, 29, 20, and 1.5 ng/mL, respectively. The odds ratio for hypothyroidism (n = 39) was 1.54 [95% confidence interval (CI): 1.00, 2.37] for an interquartile range (IQR) contrast of 13 to 68 ng/mL in serum PFOA measured in 2005-2006. However, an IQR shift in serum PFOA was not associated with TSH or TT4 levels in all children combined. IQR shifts in serum PFOS (15 to 28 ng/mL) and serum PFNA (1.2 to 2.0 ng/mL) were both associated with a 1.1% increase in TT4 in children 1-17 years old (95% CIs: 0.6, 1.5 and 0.7, 1.5 respectively). CONCLUSIONS: This is the first large-scale report in children suggesting associations of serum PFOS and PFNA with thyroid hormone levels and of serum PFOA and hypothyroidism.

Perfluorooctanoic acid exposure and pregnancy outcome in a highly exposed community.

Savitz D. A., Stein C. R., Bartell S. M., Elston B., Gong J., Shin H. M. and Wellenius G. A.

Epidemiology. 2012;23:386-92.

BACKGROUND: We assessed the association between perfluorooctanoic acid (PFOA) and pregnancy outcome in an area with elevated exposure to PFOA from drinking water contaminated by chemical plant releases.
METHODS: Serum PFOA was measured, and reproductive and residential histories were obtained during 2005-2006. We estimated serum PFOA levels at the time of pregnancy for 11,737 pregnancies occurring between 1990 and 2006, based on historical information on PFOA releases, environmental distribution, pharmacokinetic modeling, and residential histories. We assessed the association between PFOA and the odds of miscarriage, stillbirth, preeclampsia, preterm birth, term low birthweight, and birth defects, controlling for calendar time, age, parity, education, and smoking. PFOA exposure was evaluated as a continuous measure (with and without log transformation) and in quintiles, combining the lowest 2 quintiles (< 6.8 ng/mL) as the referent.
RESULTS: Measures of association between PFOA and miscarriage, preterm birth, term low birthweight, and birth defects were close to the null. Odds of stillbirth were elevated in the fourth quintile only. For preeclampsia, the odds ratio was 1.13 (95% confidence interval = 1.00-1.28) for an interquartile shift in log-transformed PFOA, and the odds ratios were 1.1-1.2 across the upper 3 quintiles of exposure.
CONCLUSIONS: In this large, population-based study in a region with markedly elevated PFOA exposure, we found no associations between estimated serum PFOA levels and adverse pregnancy outcomes other than possibly preeclampsia.

Conclusions are tempered by inherent limitations in exposure reconstruction and self-reported pregnancy outcome information.

Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome.
Stein C. R., Savitz D. A. and Dougan M.
Am J Epidemiol. 2009;170:837-46.

The authors examined the association of serum perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with self-reported pregnancy outcome in Mid-Ohio Valley residents (2000-2006) highly exposed to PFOA. Data on 1,845 pregnancies within the 5 years preceding exposure measurement were analyzed for PFOA, and data on 5,262 pregnancies were analyzed for PFOS. Generalized estimating equations were used to calculate adjusted odds ratios and 95% confidence intervals. Neither PFOA nor PFOS showed any association with miscarriage or preterm birth. Preeclampsia was weakly associated with PFOA (adjusted odds ratio = 1.3, 95% confidence interval: 0.9, 1.9) and PFOS (adjusted odds ratio = 1.3, 95% confidence interval: 1.1, 1.7) exposures above the median. PFOA was not associated with an increase in low birth weight, but PFOS showed an increased risk above the median (adjusted odds ratio = 1.5, 95% confidence interval: 1.1, 1.9) and a dose-response gradient. Birth defects were weakly associated with PFOA exposures above the 90th percentile (adjusted odds ratio = 1.7, 95% confidence interval: 0.8, 3.6). This study identified modest associations of PFOA with preeclampsia and birth defects and of PFOS with preeclampsia and low birth weight, but associations were small, limited in precision, and based solely on self-reported health outcomes.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during Pregnancy and the Birth Weight of their Offspring: The Hokkaido Study.
Kishi R., Nakajima T., Goudarzi H., Kobayashi S., Sasaki S., Okada E., Miyashita C., Itoh S., Araki A., Ikeno T., Iwasaki Y. and Nakazawa H.
Environ Health Perspect. 2015. Apr 3. [Epub ahead of print]

BACKGROUND: Fatty acids (FAs) are essential for fetal growth. Exposure to perfluorinated chemicals (PFCs) may disrupt FA homeostasis, but there is no

epidemiological data regarding associations of PFCs and FA concentrations. OBJECTIVES: We estimated associations between perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) concentrations and maternal levels of FAs and triglyceride (TG) and birth size of the offspring. METHODS: 306 mother-child pairs were analyzed in this birth cohort between 2002 and 2005 in Japan. The prenatal PFOS and PFOA levels were measured in maternal serum samples by liquid chromatography-tandem mass spectrometry. Maternal blood levels of 9 FAs and TG were measured by gas chromatography-mass spectrometry and TG-IE kits, respectively. Information of infants' birth size were obtained from participant medical records. RESULTS: The median PFOS and PFOA levels were 5.6 and 1.4 ng/mL, respectively. In the fully adjusted model, including maternal age, parity, annual household income, blood sampling period, alcohol consumption and smoking during pregnancy, PFOS, not PFOA, had a negative association with the levels of palmitic, palmitoleic, oleic, linoleic, α-linolenic, and arachidonic acids ($p < 0.005$) and TG (p value=0.016). Females weighed 186.6 g less in mothers whose PFOS levels were in the fourth quartile compared to the first quartile (95% CI: -363.4, -9.8). We observed no significant association between maternal levels of PFOS and birth weight of male infants. CONCLUSIONS: Our data suggest an inverse association between PFOS exposure and polyunsaturated FA levels in pregnant women. We also found a negative association between maternal PFOS levels and female birth weight.

Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort.

Liew Z., Ritz B., von Ehrenstein O. S., Bech B. H., Nohr E. A., Fei C., Bossi R., Henriksen T. B., Bonefeld-Jorgensen E. C. and Olsen J.
Environ Health Perspect. 2015;123:367-73.

BACKGROUND: Perfluoroalkyl substances (PFASs) are persistent pollutants found to be endocrine disruptive and neurotoxic in animals. Positive correlations between PFASs and neurobehavioral problems in children were reported in cross-sectional data, but findings from prospective studies are limited.
OBJECTIVES: We investigated whether prenatal exposure to PFASs is associated with attention deficit/hyperactivity disorder (ADHD) or childhood autism in children. METHODS: Among 83,389 mother-child pairs enrolled in the Danish National Birth Cohort during 1996-2002, we identified 890 ADHD cases and 301 childhood autism cases from the Danish National Hospital Registry and the Danish Psychiatric Central Registry. From this cohort, we randomly selected 220 cases each of ADHD and autism, and we also randomly selected 550 controls frequency matched by child's sex.

Sixteen PFASs were measured in maternal plasma collected in early or mid-pregnancy. We calculated risk ratios (RRs) using generalized linear models, taking into account sampling weights.
RESULTS: Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were detected in all samples; four other PFASs were quantified in ≥ 90% of the samples. We did not find consistent evidence of associations between mother's PFAS plasma levels and ADHD [per natural log nanograms per milliliter increase: PFOS RR = 0.87 (95% CI: 0.74, 1.02); PFOA RR = 0.98 (95% CI: 0.82, 1.16)] or autism [per natural log nanograms per milliliter increase: PFOS RR = 0.92 (95% CI: 0.69, 1.22); PFOA RR = 0.98 (95% CI: 0.73, 1.31)]. We found positive as well as negative associations between higher PFAS quartiles and ADHD in models that simultaneously adjusted for all PFASs, but these estimates were imprecise.
CONCLUSIONS: In this study we found no consistent evidence to suggest that prenatal PFAS exposure increases the risk of ADHD or childhood autism in children.

First year growth in relation to prenatal exposure to endocrine disruptors - a Dutch prospective cohort study.
de Cock M., de Boer M. R., Lamoree M., Legler J. and van de Bor M.
Int J Environ Res Public Health. 2014;11(7):7001-21.

Growth in the first year of life may already be predictive of obesity later in childhood. The objective was to assess the association between prenatal exposure to various endocrine disrupting chemicals (EDCs) and child growth during the first year. Dichloro-diphenyldichloroethylene (DDE), mono(2-ethyl-5-carboxypentyl)phthalate (MECPP), mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP), mono(2-ethyl-5-oxohexyl)phthalate (MEOHP), polychlorinated biphenyl-153, perfluorooctanesulfonic acid, and perfluoro-octanoic acid were measured in cord plasma or breast milk. Data on weight, length, and head circumference (HC) until 11 months after birth was obtained from 89 mother-child pairs. Mixed models were composed for each health outcome and exposure in quartiles. For MEOHP, boys in quartile 1 had a higher BMI than higher exposed boys ($p = 0.029$). High DDE exposure was associated with low BMI over time in boys (0.8 kg/m2 difference at 11 m). Boys with high MECPP exposure had a greater HC (1.0 cm difference at 11 m) than other boys ($p = 0.047$), as did girls in the second quartile of MEHHP ($p = 0.018$) and DDE ($p < 0.001$) exposure. In conclusion, exposure to phthalates and DDE was associated with BMI as well as with HC during the first year after birth. These results should be interpreted with caution though, due to the limited sample size.

Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood.
Ode A., Kallen K., Gustafsson P., Rylander L., Jonsson B. A., Olofsson P., Ivarsson S. A., Lindh C. H. and Rignell-Hydbom A.
PLoS One. 2014;9(4):e95891.

BACKGROUND: The association between exposure to perfluorinated compounds (PFCs) and attention deficit hyperactivity disorder (ADHD) diagnosis has been sparsely investigated in humans and the findings are inconsistent. OBJECTIVES: A matched case-control study was conducted to investigate the association between fetal exposure to PFCs and ADHD diagnosis in childhood. METHODS: The study base comprised children born in Malmo, Sweden, between 1978 and 2000 that were followed up until 2005. Children with ADHD (n = 206) were identified at the Department of Child and Adolescent Psychiatry. Controls (n = 206) were selected from the study base and were matched for year of birth and maternal country of birth. PFC concentrations were measured in umbilical cord serum samples. The differences of the PFC concentrations between cases and controls were investigated using Wilcoxon's paired test. Possible threshold effects (above the upper quartile for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and above limit of detection [LOD] for perfluorononanoic acid (PFNA)) were evaluated by conditional logistic regression. RESULTS: The median umbilical cord serum concentrations of PFOS were 6.92 ng/ml in the cases and 6.77 ng/ml in the controls. The corresponding concentrations of PFOA were 1.80 and 1.83 ng/ml. No associations between PFCs and ADHD were observed. Odds ratios adjusted for smoking status, parity, and gestational age were 0.81 (95% confidence interval [CI] 0.50 to 1.32) for PFOS, 1.07 (95% CI 0.67 to 1.7) for PFOA, and 1.1 (95% CI 0.75 to 1.7) for PFNA. CONCLUSIONS: The current study revealed no support for an association between fetal exposure to PFOS, PFOA, or PFNA and ADHD.

Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up.
Strom M., Hansen S., Olsen S. F., Haug L. S., Rantakokko P., Kiviranta H. and Halldorsson T. I.
Environ Int. 2014;68:41-8.

Fetal exposure to persistent organic pollutants (POPs) has been linked to adverse neurodevelopment, but few studies have had follow-up beyond childhood. The purpose of this study was to examine the association of maternal serum concentrations of two perfluoroalkyl acids (perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate

(PFOS)), polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (p,p'-DDE) and hexachlorobenzene (HCB) with offspring behavioural and affective disorders and scholastic achievement in a prebirth cohort study with 20 years of follow up. Between 1988 and 1989 pregnant women (n=965) were recruited for the prebirth Danish Fetal Origins 1988 (DaFO88) Cohort in Aarhus, Denmark. Perfluoroalkyl acids, PCBs, p,p'-DDE, and HCB were quantified in serum from week 30 of gestation (n=876 for perfluoroalkyl acids/872 for PCBs, p,p'-DDE, HCB). Offspring were followed up through national registries until 2011. We evaluated associations between maternal serum concentrations of these POPs and offspring neurodevelopmental outcomes, defined as: first admission diagnosis or prescription of medication until age >20 for (1) ADHD; (2) depression; and (3) scholastic achievement defined as mean grade on a standardized written examination given in the 9th grade (final exams of compulsory school in Denmark). Maternal concentrations of organochlorine substances and perfluoroalkyl acids were higher than present day levels. During the follow-up period there were 27 (3.1%) cases of ADHD and 104 (11.9%) cases of depression; the mean scholastic achievement was 6.7 (SD 2.3). Overall we found no association for maternal levels of any of the measured pollutants with offspring behavioural and affective disorders or with scholastic achievement. Our analyses based on biomarkers from a cohort of over 800 pregnant women with long-term close to complete follow-up through national registries showed little evidence of a programming effect of PFOA, PFOS, PCBs, p,p'-DDE, and HCB in relation to clinically and functionally relevant offspring neurodevelopmental outcomes.

PFOA and PFOS serum levels and miscarriage risk.
Darrow L. A., Howards P. P., Winquist A. and Steenland K.
Epidemiology. 2014;25:505-12.

BACKGROUND: Serum concentrations of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were assessed in relation to miscarriage in a population of mid-Ohio River Valley residents highly exposed to PFOA through contaminated drinking water.
METHODS: Serum PFOA and PFOS concentrations were measured in 1129 women in 2005-2006 who reported pregnancy outcomes in follow-up interviews between 2008 and 2011. In the analysis, we included 1438 reported live births, stillbirths, and miscarriages with estimated conception dates after the serum measurements. Preconception serum levels of PFOA and PFOS were analyzed in relation to miscarriage using logistic regression and generalized estimating equations.
RESULTS: There was little evidence of association between PFOA and miscarriage. For PFOS, when including all reported prospective pregnancies, the odds ratio of

miscarriage per log ng/ml increase was 1.21 (95% confidence interval = 0.94-1.55); in subanalyses restricted to each woman's first pregnancy conceived after the serum measurement, the odds ratio was 1.34 (1.02-1.76). Categorical analyses showed elevated odds ratios for the top 4 quintiles relative to the first quintile, without a monotonic trend. Positive associations between PFOS and miscarriage were strongest among nulligravid pregnancies.
CONCLUSIONS: In this prospective study of miscarriage in a population exposed to high levels of PFOA and background levels of PFOS, we found little evidence of association with serum levels of PFOA and limited evidence of association with serum levels of PFOS.

Exposure to perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations.
Leter G., Consales C., Eleuteri P., Uccelli R., Specht I. O., Toft G., Moccia T., Budillon A., Jonsson B. A., Lindh C. H., Giwercman A., Pedersen H. S., Ludwicki J. K., Zviezdai V., Heederik D., Bonde J. P. and Spano M.
Environ Mol Mutagen. 2014;55:591-600.

Perfluoroalkyl substances (PFASs) are widely used in a variety of industrial processes and products, and have been detected globally in humans and wildlife. PFASs are suspected to interfere with endocrine signaling and to adversely affect human reproductive health. The aim of the present study was to investigate the associations between exposure to PFASs and sperm global methylation levels in a population of non-occupationally exposed fertile men. Measurements of PFASs in serum from 262 partners of pregnant women from Greenland, Poland and Ukraine, were also carried out by liquid chromatography tandem mass spectrometry. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) were detected in 97% of the blood samples. Two surrogate markers were used to assess DNA global methylation levels in semen samples from the same men: (a) average DNA methylation level in repetitive DNA sequences (Alu, LINE-1, Satα) quantified by PCR-pyrosequencing after bisulfite conversion; (b) flow cytometric immunodetection of 5-methyl-cytosines. After multivariate linear regression analysis, no major consistent associations between PFASs exposure and sperm DNA global methylation endpoints could be detected. However, since weak but statistically significant associations of different PFASs with DNA hypo- and hyper-methylation were found in some of the studied populations, effects of PFASs on sperm epigenetic processes cannot be completely excluded, and this issue warrants further investigation.

Perfluoroalkyl substances during pregnancy and validated preeclampsia among nulliparous women in the Norwegian Mother and Child Cohort Study.
Starling A. P., Engel S. M., Richardson D. B., Baird D. D., Haug L. S., Stuebe A. M., Klungsoyr K., Harmon Q., Becher G., Thomsen C., Sabaredzovic A., Eggesbo M., Hoppin J. A., Travlos G. S., Wilson R. E., Trogstad L. I., Magnus P. and Longnecker M. P.
Am J Epidemiol. 2014;179:824-33.

Perfluoroalkyl substances (PFAS) are persistent and ubiquitous environmental contaminants, and human exposure to these substances may be related to preeclampsia, a common pregnancy complication. Previous studies have found serum concentrations of PFAS to be positively associated with pregnancy-induced hypertension and preeclampsia in a population with high levels of exposure to perfluorooctanoate. Whether this association exists among pregnant women with background levels of PFAS exposure is unknown. Using data from the Norwegian Mother and Child Cohort Study conducted by the Norwegian Institute of Public Health, we carried out a study of nulliparous pregnant women enrolled in 2003-2007 (466 cases, 510 noncases) to estimate associations between PFAS concentrations and an independently validated diagnosis of preeclampsia. We measured levels of 9 PFAS in maternal plasma extracted midpregnancy; statistical analyses were restricted to 7 PFAS that were quantifiable in more than 50% of samples. In proportional hazards models adjusted for maternal age, prepregnancy body mass index (weight (kg)/height (m)(2)), educational level, and smoking status, we observed no strongly positive associations between PFAS levels and preeclampsia. We found an inverse association between preeclampsia and the highest quartile of perfluoroundecanoic acid concentration relative to the lowest quartile (hazard ratio = 0.55, 95% confidence interval: 0.38, 0.81). Overall, our findings do not support an increased risk of preeclampsia among nulliparous Norwegian women with background levels of PFAS exposure.

Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study.

Braun J. M., Kalkbrenner A. E., Just A. C., Yolton K., Calafat A. M., Sjodin A., Hauser R., Webster G. M., Chen A. and Lanphear B. P.

Environ Health Perspect. 2014;122:513-20.

BACKGROUND: Endocrine-disrupting chemicals (EDCs) may be involved in the etiology of autism spectrum disorders, but identifying relevant chemicals within mixtures of EDCs is difficult.

OBJECTIVE: Our goal was to identify gestational EDC exposures associated with autistic behaviors.

METHODS: We measured the concentrations of 8 phthalate metabolites, bisphenol A, 25 polychlorinated biphenyls (PCBs), 6 organochlorine pesticides, 8 brominated flame retardants, and 4 perfluoroalkyl substances in blood or urine samples from 175 pregnant women in the HOME (Health Outcomes and Measures of the Environment) Study (Cincinnati, OH). When children were 4 and 5 years old, mothers completed the Social Responsiveness Scale (SRS), a measure of autistic behaviors. We examined confounder-adjusted associations between 52 EDCs and SRS scores using a two-stage hierarchical analysis to account for repeated measures and confounding by correlated EDCs.

RESULTS: Most of the EDCs were associated with negligible absolute differences in SRS scores (≤ 1.5). Each 2-SD increase in serum concentrations of polybrominated diphenyl ether-28 (PBDE-28) (β = 2.5; 95% CI: -0.6, 5.6) or trans-nonachlor (β = 4.1; 95% CI: 0.8-7.3) was associated with more autistic behaviors. In contrast, fewer autistic behaviors were observed among children born to women with detectable versus nondetectable concentrations of PCB-178 (β = -3.0; 95% CI: -6.3, 0.2), β-hexachlorocyclohexane (β = -3.3; 95% CI: -6.1, -0.5), or PBDE-85 (β = -3.2; 95% CI: -5.9, -0.5). Increasing perfluorooctanoate (PFOA) concentrations were also associated with fewer autistic behaviors (β = -2.0; 95% CI: -4.4, 0.4).

CONCLUSIONS: Some EDCs were associated with autistic behaviors in this cohort, but our modest sample size precludes us from dismissing chemicals with null associations. PFOA, β-hexachlorocyclohexane, PCB-178, PBDE-28, PBDE-85, and trans-nonachlor deserve additional scrutiny as factors that may be associated with childhood autistic behaviors.

No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland.

Vesterholm Jensen D., Christensen J., Virtanen H. E., Skakkeb'k N. E., Main K. M., Toppari J., Veje C. W., Andersson A. M., Nielsen F., Grandjean P. and Jensen T. K. Reproduction. 2014;147(4):411-7.

Geographical differences in the occurrence of diseases in male reproductive organs, including malformation in reproductive tract, between Denmark and Finland have been reported. The reason for these differences is unknown, but differences in exposure to chemicals with endocrine-disrupting abilities have been suggested. Among these chemicals are perfluoro-alkylated substances (PFASs), a group of water- and grease-repellent chemicals used in outdoor clothes, cookware, food packaging, and textiles. In this study, we, therefore, investigated differences in PFAS exposure levels between Denmark and Finland and the association between cord blood PFAS levels and congenital cryptorchidism. Boys from a joint ongoing prospective birth cohort study were included. We analyzed PFAS levels in cord blood serum samples collected from 29 Danish boys with congenital cryptorchidism, 30 healthy Danish matched controls recruited from 1997 to 2001, 30 Finnish cases, and 78 Finnish healthy matched controls recruited from 1997 to 1999. Additionally, 48 Finnish cases recruited from 2000 to 2002 were included. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) were detected in all the 215 Danish and Finnish cord blood samples with significantly higher levels being observed in the Danish samples (medians: PFOA, 2.6 ng/ml and PFOS, 9.1ng/ml) than in the Finnish samples (medians: PFOA, 2.1ng/ml and PFOS, 5.2ng/ml). We found no associations between cord blood PFOA and PFOS levels and congenital cryptorchidism after adjustment for confounders. Our data indicate that women in Denmark and Finland are generally exposed to PFOA and PFOS but there are differences in exposure levels between countries. We found no statistically significant association between cord blood PFOA and PFOS levels and congenital cryptorchidism; however, our study was small and larger studies are warranted.

Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study.
Wang Y., Starling A. P., Haug L. S., Eggesbo M., Becher G., Thomsen C., Travlos G., King D., Hoppin J. A., Rogan W. J. and Longnecker M. P.
Environ Health. 2013;12(1):76.

BACKGROUND: Perfluoroalkyl substances (PFASs) are a group of highly persistent chemicals that are widespread contaminants in wildlife and humans. Exposure to PFAS affects thyroid homeostasis in experimental animals and possibly in humans. The objective of this study was to examine the association between plasma concentrations of PFASs and thyroid stimulating hormone (TSH) among pregnant women. METHODS: A total of 903 pregnant women who enrolled in the Norwegian Mother and Child Cohort Study from 2003 to 2004 were studied. Concentrations of thirteen PFASs and TSH were measured in plasma samples collected around the 18th week of gestation. Linear regression models were used to evaluate associations between PFASs and TSH. RESULTS: Among the thirteen PFASs, seven were detected in more than 60% of samples and perfluorooctane sulfonate (PFOS) had the highest concentrations (median, 12.8 ng/mL; inter-quartile range [IQR], 10.1 -16.5 ng/mL). The median TSH concentration was 3.5 (IQR, 2.4 - 4.8) muIU/mL. Pregnant women with higher PFOS had higher TSH levels. After adjustment, with each 1 ng/mL increase in PFOS concentration, there was a 0.8% (95% confidence interval: 0.1%, 1.6%) rise in TSH. The odds ratio of having an abnormally high TSH, however, was not increased, and other PFASs were unrelated to TSH. CONCLUSIONS: Our results suggest an association between PFOS and TSH in pregnant women that is small and may be of no clinical significance.

The influence of endocrine disruptors in a selected population of infertile women.
Caserta D., Bordi G., Ciardo F., Marci R., La Rocca C., Tait S., Bergamasco B., Stecca L., Mantovani A., Guerranti C., Fanello E. L., Perra G., Borghini F., Focardi S. E. and Moscarini M.
Gynecol Endocrinol. 2013;29(5):444-7.

Several studies report that endocrine disrupting chemicals (EDC) able to interfere with endocrine homeostasis may affect women's reproductive health. We analyzed EDC serum levels and nuclear receptors (NRs) expression in order to have an indication of the internal dose of biologically active compounds and a measurement of indicators of their effects, as a result of the repeated uptake from environmental source. The percentage of patients with detectable bisphenol A (BPA) concentrations was

significantly higher in the infertile patients compared with fertile subjects. No significant difference was found between the groups with regard to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), mono-ethylhexyl phthalate (MEHP) and di-(2-ethylhexyl) phthalate (DEHP) concentrations. Among infertile women, the mean expression of estrogen receptor α (ERα) and beta (Erβ), androgen receptor (AR) and pregnane X receptor (PXR) was significantly higher than fertile patients. The mean expression of aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor γ (PPARγ) did not show significant differences between two groups. Patients with endometriosis had higher levels of PPARγ than all women with other causes of infertility. This study led further support to EDC exposure as a risk factor for women's fertility.

The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults.
Lin C. Y., Wen L. L., Lin L. Y., Wen T. W., Lien G. W., Hsu S. H., Chien K. L., Liao C. C., Sung F. C., Chen P. C. and Su T. C.
J Hazard Mater. 2013;244-245:637-44.

Perfluorinated chemicals (PFCs) have been widely used in a variety of products worldwide for years. However, the effect of PFCs on thyroid function has not yet been clearly defined. We recruited 567 subjects (aged 12-30 years) in a population-based cohort of adolescents and young adults with abnormal urinalysis in the childhood to determine the relationship between serum level of PFCs and the levels of serum free thyroxine (T4) and thyroid stimulating hormone (TSH). The geometric means and geometric standard deviation concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) were 2.67 (2.96) ng/ml, 7.78 (2.42) ng/ml, 1.01 (3.48) ng/ml and 5.81 (2.92) ng/ml, respectively. Differences in the levels of free T4 and TSH across different categories of PFOA, PFOS and PFUA were insignificant. After controlling for confounding factors, multiple linear regression analyses revealed mean serum level of free T4 increased significantly across categories (<60th, 60-89 and >90th percentiles) of PFNA (P for trend =0.012 in the full model). The association between PFNA and free T4 was more significant in male subjects in age group 20-30, active smokers and in those with higher body mass index in stratified analysis. Serum concentrations of PFNA were associated with serum free T4 levels in adolescents and young adults.

Persistent environmental pollutants and couple fecundity: the LIFE study.
Buck Louis G. M., Sundaram R., Schisterman E. F., Sweeney A. M., Lynch C. D., Gore-Langton R. E., Maisog J., Kim S., Chen Z. and Barr D. B.
Environ Health Perspect. 2013;121(2):231-6.

BACKGROUND: Evidence suggesting that persistent environmental pollutants may be reproductive toxicants underscores the need for prospective studies of couples for whom exposures are measured. OBJECTIVES: We examined the relationship between selected persistent pollutants and couple fecundity as measured by time to pregnancy. METHODS: A cohort of 501 couples who discontinued contraception to become pregnant was prospectively followed for 12 months of trying to conceive or until a human chorionic gonadotrophin (hCG) test confirmed pregnancy. Couples completed daily journals on lifestyle and provided biospecimens for the quantification of 9 organochlorine pesticides, 1 polybrominated biphenyl, 10 polybrominated diphenyl ethers, 36 polychlorinated biphenyls (PCBs), and 7 perfluorochemicals (PFCs) in serum. Using Cox models for discrete time, we estimated fecundability odds ratios (FORs) and 95% CIs separately for each partner's concentrations adjusting for age, body mass index, serum cotinine, serum lipids (except for PFCs), and study site (Michigan or Texas); sensitivity models were further adjusted for left truncation or time off of contraception (≤ 2 months) before enrollment. RESULTS: The adjusted reduction in fecundability associated with standard deviation increases in log-transformed serum concentrations ranged between 18% and 21% for PCB congeners 118, 167, 209, and perfluorooctane sulfonamide in females; and between 17% and 29% for p,p -DDE and PCB congeners 138, 156, 157, 167, 170, 172, and 209 in males. The strongest associations were observed for PCB 167 (FOR 0.79; 95% CI: 0.64, 0.97) in females and PCB 138 (FOR = 0.71; 95% CI: 0.52, 0.98) in males. CONCLUSIONS: In this couple-based prospective cohort study with preconception enrollment and quantification of exposures in both female and male partners, we observed that a subset of persistent environmental chemicals were associated with reduced fecundity.

Perfluorooctanoate and neuropsychological outcomes in children.
Stein C. R., Savitz D. A. and Bellinger D. C.
Epidemiology. 2013;24:590-9.

BACKGROUND: In animal studies, perfluorinated compounds affect fetal growth, development, viability, and postnatal growth. The limited epidemiologic findings on child neurobehavioral development are mixed.
METHODS: We recruited and evaluated 320 children who participated in the C8 Health Project, a 2005-2006 survey in a Mid-Ohio Valley community highly exposed to

perfluorooctanoate (PFOA) through contaminated drinking water. We examined associations among estimated in utero PFOA exposure, measured childhood PFOA serum concentration, and subsequent performance on neuropsychological tests 3-4 years later at ages 6-12 years. We assessed Intelligence Quotient (IQ) reading and math skills, language, memory and learning, visual-spatial processing, and attention. All multivariable linear regression models were adjusted for age, sex, home environment, test examiner, and maternal IQ. Models with measured childhood PFOA were additionally adjusted for child body mass index.
RESULTS: Children in the highest as compared with lowest quartile of estimated in utero PFOA had increases in Full Scale IQ (β 4.6, 95% confidence interval [CI] = 0.7-8.5) and decreases in characteristics of attention deficit/hyperactivity disorder as measured by the Clinical Confidence Index of Connors' Continuous Performance Test-II β -8.5, 95% CI = -16.1 to -0.8). There were negligible associations between PFOA and reading or math skills or neuropsychological functioning.
CONCLUSION: These results do not suggest an adverse association between the levels of PFOA exposure experienced by children in this cohort and their performance on neuropsychological tests.

Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age.
Chen M. H., Ha E. H., Liao H. F., Jeng S. F., Su Y. N., Wen T. W., Lien G. W., Chen C. Y., Hsieh W. S. and Chen P. C.
Epidemiology. 2013;24:800-8.

BACKGROUND: Epidemiologic data regarding the potential neurotoxicity of perfluorinated compounds (PFCs) are inconclusive. We investigated the associations between in utero exposure to perfluorooctanoic acid (PFOA) and perfluorooctyl sulfonate (PFOS) and early childhood neurodevelopment.
METHODS: We recruited 239 mother-infant pairs in northern Taiwan from the Taiwan Birth Panel Study, which was established in 2004. We examined the association between PFCs in cord blood and children's neurodevelopment at 2 years of age, using the Comprehensive Developmental Inventory for Infants and Toddlers. This tool contains cognitive, language, motor, social, and self-help domains; test scores were further transformed into developmental quotients according to standardized norms. All multivariate regression models were adjusted for infant sex and gestational age, maternal education, family income, cord blood cotinine levels, postnatal environmental tobacco smoke exposure, and breastfeeding.
RESULTS: Prenatal PFOS concentrations in both untransformed and natural log (Ln)-transformed values were associated with adverse performance on the whole test and

the domains related to development. A dose-response relationship was observed when PFOS levels were categorized into four groups. This association was most obvious in relation to the gross-motor subdomain. Across the PFOS interquartile range, the quotients of the gross-motor subdomain decreased by 3.7 points (95% confidence interval [CI] = -6.0 to -1.5), with an increasing odds ratio of poor performance (2.4; 95% CI = 1.3 to 4.2). In contrast, measures of association between PFOA concentrations and test scores were close to null.
CONCLUSIONS: Prenatal exposure to PFOS, but not PFOA, may affect children's development, especially gross-motor development at 2 years of age.

Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age.
Andersen C. S., Fei C., Gamborg M., Nohr E. A., Sorensen T. I. and Olsen J.
Am J Epidemiol. 2013;178:921-7.

Fetal exposure to the perfluoroalkyl acids, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA), has been associated with lower birth weight and lower weight and body mass index (weight (kg)/height (m)(2)) in early infancy. It is, however, unclear if exposure to prenatal PFOS and PFOA has a lasting influence on growth. We estimated the associations between the maternal plasma level of PFOS or PFOA and the children's body mass index, waist circumference, and risk of overweight at 7 years of age. A total of 1,400 women were randomly selected from the Danish National Birth Cohort among those who provided blood samples early in pregnancy and gave birth to liveborn singletons in 1996-2002. Weight and height information at 7 years was available for 811 children. Multiple linear and logistic regression models were used for analyses. Maternal PFOS and PFOA concentrations were overall inversely but nonsignificantly associated with the children's body mass index, waist circumference, and risk of overweight at 7 years of age. In conclusion, plasma levels of PFOS and PFOA in pregnant women did not seem to have any appreciable influence on their children's anthropometry at this point in childhood.

Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland.
Kvist L., Giwercman Y. L., Jonsson B. A., Lindh C. H., Bonde J. P., Toft G., Strucinski P., Pedersen H. S., Zvyezday V. and Giwercman A.
Reprod Toxicol. 2012;34(4):644-50.

This study investigated whether perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), which exhibit reproductive toxicity in experimental

animals, affect sperm sex chromosome ratio. The Y:X ratio was determined by fluorescence in situ hybridization. Serum concentrations of PFOA and PFOS were measured in 607 men from Greenland, Poland and Ukraine using liquid chromatography-tandem mass spectrometry. Data was analyzed by linear and nonlinear regression. We observed no associations between PFOA and Y:X ratio (p=0.845 in a linear model, p=0.296 in a nonlinear model). A positive nonlinear association between PFOS and Y:X ratio was observed (p=0.016), with no association in a linear model (p=0.118). Analyzing the populations separately, a negative trend between categorized PFOS exposure and Y:X ratio was observed for the Inuit (B=-0.002, p=0.044). In conclusion, there was a negative trend between Y:X ratio and PFOS in the Inuit, while there was no association between PFOA and the Y:X ratio in adult men.

Perfluorinated compounds in umbilical cord blood and adverse birth outcomes.
Chen M. H., Ha E. H., Wen T. W., Su Y. N., Lien G. W., Chen C. Y., Chen P. C. and Hsieh W. S.
PLoS One. 2012;7(8):e42474.

BACKGROUND: Previous animal studies have shown that perfluorinated compounds (PFCs) have adverse impacts on birth outcomes, but the results have been inconclusive in humans. We investigated associations between prenatal exposure to perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA) and birth outcomes. METHODS: In total, 429 mother-infant pairs were recruited from the Taiwan Birth Panel Study (TBPS). Demographic data were obtained by interviewing mothers using a structured questionnaire and birth outcomes were extracted from medical records. Cord blood was collected for PFOA, PFOS, PFNA, and PFUA analysis by ultra-high-performance liquid chromatography/tandem mass spectrometry. RESULTS: The geometric mean (standard deviation) levels of PFOA, PFOS, PFNA, and PFUA in cord blood plasma were 1.84 (2.23), 5.94 (1.95), 2.36(4.74), and 10.26 (3.07) ng/mL, respectively. Only PFOS levels were found to be inversely associated with gestational age, birth weight, and head circumference [per ln unit: adjusted β (95% confidence interval, CI) = -0.37 (-0.60, -0.13) wks, -110.2 (-176.0, -44.5) gm and -0.25 (-0.46, -0.05) cm]. Additionally, the odds ratio of preterm birth, low birth weight, and small for gestational age increased with PFOS exposure [per ln unit: adjusted odds ratio (OR) (95%CI) = 2.45 (1.47, 4.08), 2.61(0.85, 8.03) and 2.27 (1.25, 4.15)]. When PFOS levels were divided into quartiles, a dose-response relation was observed. However, PFOA, PFNA, and PFUA were not observed to have any convincing impact on birth outcomes. CONCLUSIONS: An adverse dose-dependent association was observed between

prenatal PFOS exposure and birth outcomes. However, no associations were found for the other examined PFCs.

Exposure to perfluorinated compounds and human semen quality in Arctic and European populations.
Toft G., Jonsson B. A., Lindh C. H., Giwercman A., Spano M., Heederik D., Lenters V., Vermeulen R., Rylander L., Pedersen H. S., Ludwicki J. K., Zviezdai V. and Bonde J. P.
Hum Reprod. 2012;27(8):2532-40.

BACKGROUND: Perfluorinated compounds (PFCs) have been suspected to adversely affect human reproductive health. The aim of this study was to investigate the associations between PFC exposure and male semen quality. METHODS: PFCs were measured in serum from 588 partners of pregnant women from Greenland, Poland and Ukraine who provided a semen sample, using liquid chromatography tandem mass spectrometry. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) could be detected in >97% of the samples. The associations between levels of these compounds and semen volume, sperm concentration, total sperm count, motility and morphology were assessed. RESULTS: Across countries, sperm concentration, total sperm count and semen volume were not consistently associated with PFOS, PFOA, PFHxS or PFNA levels. The proportion of morphologically normal cells was 35% lower [95% confidence interval (CI): 4-66%) for the third tertile of PFOS exposure as compared with the first. A similar reduction was found in relation to increasing PFHxS levels. At the third PFOA exposure tertile, the percentage of motile spermatozoa was 19% (95% CI: 1 to 39%) higher than in the first. CONCLUSIONS: The most robust finding in the present study was the negative associations between PFOS exposure and sperm morphology suggesting adverse effects of PFOS on semen quality, possibly due to interference with the endocrine activity or sperm membrane function. It cannot be excluded that this association and the positive association between PFOA and semen motility, which was not consistent across countries, might represent a chance finding due to the multiple statistical tests being performed.

Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements.
Raymer J. H., Michael L. C., Studabaker W. B., Olsen G. W., Sloan C. S., Wilcosky T. and Walmer D. K.
Reprod Toxicol. 2012;33(4):419-27.

A total of 256 men were studied to evaluate whether serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) impacted semen quality or reproductive hormones. Blood and semen were collected and analyzed for perfluorochemicals and reproductive and thyroid hormones. Semen quality was assessed using standard clinical methods. Linear and logistic modeling was performed with semen profile measurements as outcomes and PFOS and PFOA in semen and plasma as explanatory variables. Adjusting for age, abstinence, and tobacco use, there was no indication that PFOA or PFOS was significantly associated with volume, sperm concentration, percent motility, swim-up motility and concentration, and directional motility (a function of motility and modal progression). Follicle-stimulating hormone was not associated with either PFOA or PFOS. Luteinizing hormone was positively correlated with plasma PFOA and PFOS, but not semen PFOS. Important methodological concerns included the lack of multiple hormonal measurements necessary to address circadian rhythms.

Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones.
Ji K., Kim S., Kho Y., Paek D., Sakong J., Ha J., Kim S. and Choi K.
Environ Int. 2012;45:78-85.

Perfluorinated compounds (PFCs) have been frequently detected in both the environment and biota, and have become a growing concern. However, information is limited on the potential sources and human health implications of such exposure. We evaluated the exposure levels of 13 major PFCs among a population (n=633, >12 years of age) in a mid-sized city of Korea, and investigated for their potential dietary sources and the impact on thyroid hormone concentrations. For this purpose, we collected blood samples from a general population in Siheung, Korea and measured for 13 PFCs, total thyroxine (T4), and thyroid stimulating hormone (TSH). In addition, a questionnaire survey on diet was conducted. Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) were detected in relatively greater concentrations than the other 9 PFCs in the blood serum. Males tend to have greater concentrations than females for most PFCs, and the concentrations were elevated as age increased up to

50s. Body mass index (BMI) was also shown to influence the serum concentrations of several PFCs. After adjustment for age, sex, and BMI, the consumption of vegetable, potato, fish/shellfish, and popcorn was identified to be significantly related with concentrations of major PFCs in blood. Among the studied PFCs, the concentrations of perfluorotridecanoic acid (PFTrDA) were negatively correlated with total T4, and positively with TSH levels, especially among females. The result of this study will provide information useful for developing public health and safety management measures for PFCs.

Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley.
Savitz D. A., Stein C. R., Elston B., Wellenius G. A., Bartell S. M., Shin H. M., Vieira V. M. and Fletcher T.
Environ Health Perspect. 2012;120(8):1201-7.

BACKGROUND: Perfluorooctanoic acid (PFOA) is a potential cause of adverse pregnancy outcomes, but previous studies have been limited by low exposures and small study size. OBJECTIVES: Using birth certificate information, we examined the relation between estimated PFOA exposure and birth outcomes in an area of West Virginia and Ohio whose drinking water was contaminated by a chemical plant. METHODS: Births in the study area from 1990 through 2004 were examined to generate case groups of stillbirth (n = 106), pregnancy-induced hypertension (n = 224), preterm birth (n = 3,613), term low birth weight (n = 918), term small-for-gestational-age (SGA) (n = 353), and a continuous measure of birth weight among a sample of term births (n = 4,534). A 10% sample of term births >/= 2,500 g were selected as a source of controls (n = 3,616). Historical estimates of serum PFOA were derived from a previously developed fate and transport model. In a second study, we examined 4,547 area births linked to a survey with residential history data. RESULTS: In the analysis based only on birth records, we found no consistent evidence of an association between estimated PFOA exposure and stillbirth, pregnancy-induced hypertension, preterm birth, or indices of fetal growth. In the analysis of birth records linked to the survey, PFOA was unrelated to pregnancy-induced hypertension or preterm birth but showed some suggestion of an association with early preterm birth. Measures of growth restriction showed weak and inconsistent associations with PFOA. CONCLUSIONS: Based on the analysis using the health survey, these results provide little support for an effect of PFOA exposure on most pregnancy outcomes, except for early preterm birth and possibly fetal growth restriction.

Exposure and effective dose biomarkers for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in infertile subjects: preliminary results of the PREVIENI project.

La Rocca C., Alessi E., Bergamasco B., Caserta D., Ciardo F., Fanello E., Focardi S., Guerranti C., Stecca L., Moscarini M., Perra G., Tait S., Zaghi C. and Mantovani A.
Int J Hyg Environ Health. 2012;215(2):206-11.

Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been used as surfactants in various industry and consumer products. PFOS/PFOA are very persistent in the environment and bioaccumulate in humans. They are potential reproductive and developmental toxicants and are considered to be emerging endocrine disrupters (EDs). The Italian project PREVIENI, funded by the Italian Environment Ministry, aims to link environment and human health through the investigation of selected endocrine disrupters (EDs) exposure and associated biomarkers related to human infertility conditions. In the early PREVIENI phase, PFOS and PFOA were determined in 53 couples affected by an infertility status, enrolled in a metropolitan area, according to established inclusion criteria and informed consensus. Nuclear receptors related to chemical compounds interactions were selected as biomarkers of effect and their gene expression modulations were analyzed in human peripheral blood mononuclear cell (PBMC). Among couples, subjects not presenting infertility factors (IF--) were separated from affected subjects (IF++). Most IF-- serum samples showed PFOS and PFOA concentrations overlapping the limit of detection (LOD) of 0.5 ng/g wet weight (ww). A substantial percentage of IF++ serum samples showed PFOS concentrations >20-fold the LOD, i.e. from 3 to 50 ng/g ww. In male (50%, n=26) and from 3 to 144 ng/g ww in female (37%, n=30) samples. PFOA values were below the LOD levels in 90% of the total samples. Peroxisome proliferator-activated receptor-γ (PPARγ) and aryl hydrocarbon receptor (AhR) showed a low level of expression in PBMC of both IF++ and IF-- groups. Whereas α and β estrogen receptors (ERα and ERβ), androgen receptor (AR), and pregnane X receptor (PXR) were all upregulated in IF++ of both sexes with respect to IF-- group. Our preliminary results related to the metropolitan area indicate that subjects affected by infertility factors tend to have both higher PFOS levels and higher gene expression of specific nuclear receptors.

Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive.
Vestergaard S., Nielsen F., Andersson A. M., Hjollund N. H., Grandjean P., Andersen H. R. and Jensen T. K.
Hum Reprod. 2012;27:873-80.

BACKGROUND: Perfluorinated chemicals (PFCs) have been widely used and have emerged as important food contaminants. A recent study on pregnant women suggested that PFC exposure was associated with a longer time to pregnancy (TTP). We examined the association between serum concentrations of PFCs in females and TTP in 222 Danish first-time pregnancy planners during the years 1992-1995.
METHODS: The couples were enrolled in the study when discontinuing birth control and followed for six menstrual cycles or until a clinically recognized pregnancy occurred. Fecundability ratio (FR) was calculated using discrete-time survival models. In addition, odds ratio (OR) for TTP > 6 cycles was calculated.
RESULTS: OR for TTP > 6 cycles for those with PFC concentrations above the median were 0.96 [95% confidence interval (CI): 0.54-1.64] for perfluorooctane sulfonic acid (PFOS), the major PFC, compared with those below the median. FRs for those with PFOS concentrations above the median were 1.05 (95% CI: 0.74-1.48) compared with those below the median. Other PFCs showed the same lack of association with TTP. The results were not affected by adjustment for covariates. PFOS and perfluorooctanoic acid concentrations were similar to those observed in a previous Danish study.
CONCLUSIONS: These findings suggest that exposure to PFCs affects TTP only to a small extent, if at all.

Perfluorinated acids and hypothyroxinemia in pregnant women.
Chan E., Burstyn I., Cherry N., Bamforth F. and Martin J. W.
Environ Res. 2011;111(4):559-64.

Perfluorinated acids (PFAs) are prominent and widespread contaminants of human blood. In animal studies there is evidence that suggests certain PFAs can disrupt thyroid hormone homeostasis. A commonly reported condition in exposed animals is hypothyroxinemia, whereby serum free thyroxine (fT4) is decreased despite normal thyroid stimulating hormone (TSH) concentrations. We designed an individually matched case-control study to investigate whether exposure to perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) was associated with hypothyroxinemia in pregnant women from Edmonton, Alberta, Canada, in 2005-2006, who underwent a "triple screen" blood test at 15-20 weeks

gestation as part of ante-natal care. Thyroid hormones, fT4 and TSH, were measured in serum from 974 women, and from these we measured PFAs in the sera of 96 hypothyroxinemic cases (normal TSH, the lowest 10th percentile of fT4) and 175 controls (normal TSH, fT4 between the 50th and 90th percentiles) matched on age and referring physician. Analyses by conditional logistic regression indicated that the concentrations of PFAs in this population were not associated with hypothyroxinemia among pregnant women. The current findings do not support a causal link between PFA exposure and maternal hypothyroxinemia in the studied population.

Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years.
Fei C. and Olsen J.
Environ Health Perspect. 2011;119(4):573-8.

OBJECTIVE: Potential neurotoxic effects of perfluorinated compounds (PFCs) have been reported in highly exposed animals, but whether these chemicals are neurotoxic in humans is not known. We therefore investigated whether prenatal exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfate (PFOS), two of the most prevalent PFCs, are associated with behavioral or coordination problems in early childhood. METHODS: We used data from the Danish National Birth Cohort, which enrolled mothers in early pregnancy, and we measured maternal blood levels of PFOA and PFOS using specimens drawn around 8 weeks of gestation. When the children reached 7 years of age, mothers completed the Strengths and Difficulties Questionnaire (SDQ, n=787) and the Developmental Coordination Disorder Questionnaire (DCDQ, n=526) to assess behavioral health and motor coordination of their children. SDQ scores above the 90th percentile were a priori defined to identify behavioral problems and DCDQ scores below the 10th percentile were defined as a potential DCD. RESULTS: The median concentrations of PFOS and PFOA in maternal blood were 34.4 ng/mL [interquartile range (IQR), 26.6-44.5] and 5.4 ng/mL (IQR, 4.0-7.1), respectively, similar to distributions reported for populations without occupational exposure. We found no association between higher SDQ scores and maternal levels of PFOS or PFOA, nor did we see any statistically significant association with motor coordination disorders. CONCLUSION: The findings suggest that background levels of PFOA and PFOS are not associated with behavioral and motor coordination problems in childhood. However, effects on other developmental end points, including cognitive, attentional, and clinical mental disorders not measured in this study, cannot be ruled out.

Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort.
Christensen K. Y., Maisonet M., Rubin C., Holmes A., Calafat A. M., Kato K., Flanders W. D., Heron J., McGeehin M. A. and Marcus M.
Environ Int. 2011;37(1):129-35.

INTRODUCTION: Polyfluoroalkyl chemicals (PFCs) are commercially synthesized chemicals used in consumer products. Exposure to certain PFCs is widespread, and some PFCs may act as endocrine disruptors. We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom to conduct a nested case-control study examining the association between age at menarche, and exposure to PFCs during pregnancy. METHODS: Cases were selected from female offspring in the ALSPAC who reported menarche before the age of 11.5 years (n = 218), and controls were a random sample of remaining girls (n = 230). Serum samples taken from the girls' mothers during pregnancy (1991-1992) were analyzed using on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry for 8 PFCs. Logistic regression was used to determine association between maternal serum PFC concentrations, and odds of earlier age at menarche. RESULTS: PFOS and PFOA were the predominant PFCs (median serum concentrations of 19.8 ng/mL and 3.7 ng/mL). All but one PFC were detectable in most samples. Total PFC concentration varied by number of births (inverse association with birth order; p-value < 0.0001) and race of the child (higher among whites; p-value = 0.03). The serum concentrations of carboxylates were associated with increased odds of earlier age at menarche; concentrations of perfluorooctane sulfonamide, the sulfonamide esters and sulfonates were all associated with decreased odds of earlier age at menarche. However, all confidence intervals included the null value of 1.0. CONCLUSIONS: ALSPAC study participants had nearly ubiquitous exposure to most PFCs examined, but PFC exposure did not appear to be associated with altered age at menarche of their offspring.

Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones.
Kim S., Choi K., Ji K., Seo J., Kho Y., Park J., Kim S., Park S., Hwang I., Jeon J., Yang H. and Giesy J. P.
Environ Sci Technol. 2011;45:7465-72.

While the results of animal studies have shown that perfluorinated compounds (PFCs) can modulate concentrations of thyroid hormones in blood, limited information is

available on relationships between concentrations of PFCs in human blood serum and fetal thyroid hormones. The relationship between concentrations of PFCs in blood and fetal thyroid hormone concentrations or birth weight, and ratios of major PFCs between maternal and fetal serum were determined. Concentrations of PFCs were measured in blood serum of pregnant women (n = 44), fetal cord blood serum (n = 43) and breast milk (n = 35). Total concentrations of thyroxin (T4), triiodothyronin (T3) and thyroid stimulating hormone (TSH) in blood serum were also quantified. The ratios of major PFCs in maternal versus fetal serum were 1:1.93, 1.02, 0.72, and 0.48 for perfluorotridecanoic acid (PFTrDA), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS), respectively. Fetal PFOS, PFOA, PFTrDA and maternal PFTrDA were correlated with fetal total T4 concentrations, but after adjusting for major covariates, most of the relationships were no longer statistically significant. However, the significant negative correlations between maternal PFOS and fetal T3, and maternal PFTrDA and fetal T4 and T3 remained. Since thyroid hormones are crucial in the early development of the fetus, its clinical implication should be evaluated. Given the observed trans-placental transfer of PFCs, efforts should be also made to elucidate the exposure sources among pregnant women.

Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Environ Res. 2010;110(8):773-7.

OBJECTIVES: To examine whether prenatal exposure to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) is associated with the occurrence of hospitalization for infectious diseases during early childhood. METHODS: We randomly selected 1400 pregnant women and their offspring from the Danish National Birth Cohort (1996-2002) and measured PFOS and PFOA levels in maternal blood during early pregnancy. Hospitalizations for infection of the offspring were identified by the linkage to the National Hospital Discharge Register through 2008. RESULTS: Hospitalizations due to infections were not associated with prenatal exposure to PFOA and PFOS. On the contrary, the relative risks of hospitalizations ranged from 0.71 to 0.84 for the three higher quartiles of maternal PFOA levels compared with the lowest, but no dose-response pattern was found. No clear pattern was observed when results were stratified by child's age at infection, with the exception of an inverse association between maternal PFC levels and risk of hospitalization during the child's first year of life. CONCLUSIONS: These findings suggest that prenatal exposure to PFOA or

PFOS is not associated with increased risk of infectious diseases leading to hospitalization in early childhood.

Exploratory assessment of perfluorinated compounds and human thyroid function.
Bloom M. S., Kannan K., Spliethoff H. M., Tao L., Aldous K. M. and Vena J. E.
Physiol Behav. 2010;99(2):240-5.

Thyroid hormones play critical roles in human neurodevelopment and adult neurocognitive function. Persistent organohalogen pollutants, such as perfluorinated compounds (PFCs), may interfere with thyroid homeostasis and thus exposures to these compounds might represent risk factors for neurologic and cognitive abnormalities. In this study, serum specimens collected from thirty-one licensed anglers in New York State were analyzed for levels of thyroid stimulating hormone (TSH), free thyroxine (FT(4)), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA), and perfluoroundecanoic acid (PFUnDA). PFOS and PFOA occurred in the highest concentrations with geometric means of 19.6 ng/mL (95% CI 16.3-23.5) and 1.3 ng/mL (95% CI 1.2-1.5), respectively. In a cross-sectional analysis, no statistically significant associations were detected for PFCs, or their sum, with TSH or FT(4) at α=0.05. However, post hoc power analyses, though limited, suggested that moderate increases in sample size, to 86 and 129 subjects, might facilitate 80% power to detect statistically significant associations for FT(4) and PFDA (β=0.09) and PFUnDA (β=0.08), respectively. The consumption of sportfish may have contributed to PFDA (r=0.52, P=0.003) and PFUnDA (r=0.40, P=0.025) levels. This preliminary study does not indicate associations between non-occupational PFCs exposures and thyroid function. However, the possibility for weak associations for FT(4) with PFDA and PFUnDA, PFCs measured in low concentrations, is raised. Given the ubiquity of PFCs in the environment and the importance of thyroid function to neurodevelopmental and neurocognitive endpoints, a confirmatory study is warranted.

Maternal exposure to perfluorinated acids and fetal growth.
Hamm M. P., Cherry N. M., Chan E., Martin J. W. and Burstyn I.
J Expo Sci Environ Epidemiol. 2010;20:589-97.

The widespread detection of perfluorinated acids (PFAs) in humans and known developmental toxicity in animals has raised concern about their potential effects on human reproductive health. Our objective was to determine whether increasing

maternal exposure to PFAs is associated with adverse effects on fetal growth and length of gestation in women giving birth in Alberta, Canada. We examined the concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS) in a cohort of 252 pregnant women who gave birth to live singletons. Each of the women had undergone an early second trimester prenatal screen, and her serum was analyzed for PFA concentrations. Data on infant and maternal variables were collected from the delivery record completed at birth. Adjusted changes in birth weight per natural log (ng/ml) of PFOA (median 1.5 ng/ml), PFHxS (median 0.97 ng/ml), and PFOS (median 7.8 ng/ml) were -37.4 g (95% confidence interval (CI): -86.0 to 11.2 g), 21.9 g (-23.4 to 67.2 g), and 31.3 g (-43.3 to 105.9 g), respectively. Mean birth weight z-score, standardized for gestational age and gender, length of gestation, and risk of preterm birth did not appear to be influenced by maternal PFA exposure. When PFA concentrations were divided into tertiles, similar patterns were observed. These results suggest that maternal PFA exposure has no substantial effect on fetal weight and length of gestation at the concentrations observed in this population.

The relationship between birth weight, gestational age and perfluorooctanoic acid (PFOA)-contaminated public drinking water.
Nolan L. A., Nolan J. M., Shofer F. S., Rodway N. V. and Emmett E. A.
Reprod Toxicol. 2009;27(3-4):231-8.

BACKGROUND: Recent studies have examined the associations between perfluorooctanoic acid (PFOA) levels in cord blood and maternal plasma with lowered birth weight and gestational age in humans; however, no study has examined these effects in a population of known high PFOA exposure. Residents drinking PFOA-contaminated water from the Little Hocking Water Association (LHWA) in Washington County, Ohio have serum PFOA levels approximately 80 times those in the general U.S. population. OBJECTIVES: To compare birth weights and gestational ages of neonates born to mothers residing in zip codes with water service provided completely, partially or not at all by the LHWA. METHODS: Multiple logistic and linear regression analyses were performed on singleton neonatal birth weight data supplied by the Ohio Department of Health to examine the associations between LHWA water service category (used as a surrogate for PFOA exposure) with mean birth weight, mean gestational age, the likelihood of low birth weight (<2500 g), and the likelihood of preterm birth (<37 completed weeks of gestation). All models were adjusted for maternal age, gestational age, sex, race and population-level socioeconomic status. RESULTS: The incidence of low birth weight, preterm birth, mean birth weight and mean gestational age of neonates did not significantly differ among water service

categories. CONCLUSION: Markedly elevated PFOA exposure, as categorized by water service category, is not associated with increased risk of lowered birth weight or gestational age. This study does not confirm earlier findings of an association between PFOA and lowered birth weight observed at normal population levels.

Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth.
Washino N., Saijo Y., Sasaki S., Kato S., Ban S., Konishi K., Ito R., Nakata A., Iwasaki Y., Saito K., Nakazawa H. and Kishi R.
Environ Health Perspect. 2009;117:660-7.

BACKGROUND: Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are man-made, ubiquitous, and persistent contaminants in the environment, wildlife, and humans. Although recent studies have shown that these chemicals interfere with fetal growth in humans, the results are inconsistent.
OBJECTIVES: Our goal was to investigate the correlation between relatively low levels of PFOS and PFOA in maternal serum and birth weight and birth size.
METHODS: We conducted a hospital-based prospective cohort study between July 2002 and October 2005 in Sapporo, Japan. A total of 428 women and their infants were involved in the study. We obtained characteristics of the mothers and infants from self-administered questionnaire surveys and from medical records. We analyzed maternal serum samples for PFOS and PFOA by liquid chromatography-tandem mass spectrometry (LC/MS/MS).
RESULTS: After adjusting for confounding factors, PFOS levels negatively correlated with birth weight [per log10 unit: β = -148.8 g; 95% confidence interval (CI), -297.0 to -0.5 g]. In addition, analyses stratified by sex revealed that PFOS levels negatively correlated with birth weight only in female infants (per log10 unit: β = -269.4 g; 95% CI, -465.7 to -73.0 g). However, we observed no correlation between PFOA levels and birth weight.
CONCLUSION: Our results indicate that in utero exposure to relatively low levels of PFOS was negatively correlated with birth weight.

Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Environ Health Perspect. 2008;116(10):1391-5.

BACKGROUND: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are fluorinated organic compounds present in the general population at low concentrations.

Animal studies have shown that they may affect neuromuscular development at high concentrations. OBJECTIVES: We investigated the association between plasma levels of PFOS and PFOA in pregnant women and motor and mental developmental milestones of their children. METHODS: We randomly selected 1,400 pairs of pregnant women and their children from the Danish National Birth Cohort. PFOS and PFOA were measured in maternal blood samples taken in early pregnancy. Apgar score was abstracted from the National Hospital Discharge Register in Denmark. Developmental milestones were reported by mothers using highly structured questionnaires when the children were around 6 months and 18 months of age. RESULTS: Mothers who had higher levels of PFOA and PFOS gave birth to children who had similar Apgar scores and reached virtually all of the development milestones at the same time as children born to mothers with lower exposure levels. Children who were born to mothers with higher PFOS levels were slightly more likely to start sitting without support at a later age. CONCLUSION: We found no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS as measured in maternal plasma early in pregnancy.

Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples.
Monroy R., Morrison K., Teo K., Atkinson S., Kubwabo C., Stewart B. and Foster W. G. Environ Res. 2008;108(1):56-62.

Perfluoroalkyl compounds (PFCs) are end-stage metabolic products from industrial flourochemicals used in the manufacture of plastics, textiles, and electronics that are widely distributed in the environment. The objective of the present study was to quantify exposure to perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorodecanoic acid (PFDeA), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), and perfluorononanoic acid (PFNA) in serum samples collected from pregnant women and the umbilical cord at delivery. Pregnant women (n=101) presenting for second trimester ultrasound were recruited and PFC residue levels were quantified in maternal serum at 24-28 weeks of pregnancy, at delivery, and in umbilical cord blood (UCB; n=105) by liquid chromatography-mass spectrometry. Paired t-test and multiple regression analysis were performed to determine the relationship between the concentrations of each analyte at different sample collection time points. PFOA and PFOS were detectable in all serum samples analyzed including the UCB. PFOS serum levels (mean+/-S.D.) were significantly higher ($p<0.001$) in second trimester maternal serum (18.1+/-10.9 ng/mL) than maternal serum levels at delivery (16.2+/-10.4 ng/mL), which were higher than the levels found in UCB (7.3+/-5.8 ng/mL; $p<0.001$). PFHxS was quantifiable in 46/101

(45.5%) maternal and 21/105 (20%) UCB samples with a mean concentration of 4.05+/-12.3 and 5.05+/-12.9 ng/mL, respectively. There was no association between serum PFCs at any time point studied and birth weight. Taken together our data demonstrate that although there is widespread exposure to PFCs during development, these exposures do not affect birth weight.

Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth.
Apelberg B. J., Witter F. R., Herbstman J. B., Calafat A. M., Halden R. U., Needham L. L. and Goldman L. R.
Environ Health Perspect. 2007;115(11):1670-6.

BACKGROUND: Recent studies have reported developmental toxicity among rodents dosed with perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). OBJECTIVES: We examined the relationship between concentrations of PFOS and PFOA in cord serum (surrogates for in utero exposures) and gestational age, birth weight, and birth size in humans. METHODS: We conducted a hospital-based cross-sectional epidemiologic study of singleton deliveries in Baltimore, Maryland. Cord serum samples (n = 293) were analyzed for PFOS and PFOA by online solid-phase extraction, coupled with reversed-phase high-performance liquid chromatography-isotope dilution tandem mass spectrometry. Maternal characteristics and anthropometric measures were obtained from medical charts. RESULTS: After adjusting for potential confounders, both PFOS and PFOA were negatively associated with birth weight [per ln-unit: β = -69 g, 95% confidence interval (CI), -149 to 10 for PFOS; β = -104 g, 95% CI, -213 to 5 for PFOA], ponderal index (per ln-unit: β = -0.074 g/cm(3) x 100, 95% CI, -0.123 to -0.025 for PFOS; β = -0.070 g/cm(3) x 100, 95% CI, -0.138 to -0.001 for PFOA), and head circumference (per ln-unit: β = -0.32 cm, 95% CI, -0.56 to -0.07 for PFOS; β = -0.41 cm, 95% CI, -0.76 to -0.07 for PFOA). No associations were observed between either PFOS or PFOA concentrations and newborn length or gestational age. All associations were independent of cord serum lipid concentrations. CONCLUSIONS: Despite relatively low cord serum concentrations, we observed small negative associations between both PFOS and PFOA concentrations and birth weight and size. Future studies should attempt to replicate these findings in other populations.

C. Studies with unclear findings

Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES).
Taylor K. W., Hoffman K., Thayer K. A. and Daniels J. L.
Environ Health Perspect. 2014;122(2):145-50.

BACKGROUND: Polyfluoroalkyl chemicals (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been associated with early menopause. However, previous cross-sectional studies have lacked adequate data to investigate possible reverse causality (i.e., higher serum concentrations due to decreased excretion after menopause). OBJECTIVES: We investigated the association between PFOS, PFOA, perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS) and age at natural menopause among women 20-65 years of age in NHANES (National Health and Nutrition Examination Survey). METHODS: We used proportional hazard models to estimate hazard ratios (HRs) for the onset of natural menopause as a function of age and serum PFC levels, and to investigate reverse causation by estimating associations between PFC levels and the rate of hysterectomy. We also used multivariable linear regression to determine whether time since menopause predicted serum PFC levels. RESULTS: After adjusting for age at survey, race/ethnicity, education, ever smoking, and parity, women with higher levels of PFCs had earlier menopause than did women with the lowest PFC levels. We observed a monotonic association with PFHxS: The HR was 1.42 (95% CI: 1.08, 1.87) for serum concentrations in tertile 2 versus tertile 1, and 1.70 (95% CI: 1.36, 2.12) for tertile 3 versus tertile 1. We also found evidence of reverse causation: PFCs were positively associated with rate of hysterectomy, and time since natural menopause was positively associated with serum PFCs. CONCLUSIONS: Our findings suggest a positive association between PFCs and menopause; however, at least part of the association may be due to reverse causation. Regardless of underlying cause, women appear to have higher PFC concentrations after menopause.

Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women.
Jiang W., Zhang Y., Zhu L. and Deng J.
Environ Int. 2014;64:40-7.

Perfluoroalkyl acids (PFAAs) are a group of chemicals used for many applications and widely present in the environment and humans. In this study, serum levels of PFAAs

and isomers of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) were analyzed in 141 Chinese pregnant women. Among all the samples, total PFOS (Σ PFOS, mean concentration 7.32ng/mL) was predominant, followed by Σ PFOA (mean 4.78ng/mL) and perfluorodecanoate (PFDA, mean 1.45ng/mL). On average, the proportion of linear PFOS (n-PFOS) was 66.7% of Σ PFOS, which was higher than the general population, implying that maternal women could excrete branched PFOS isomers to the fetus by transplacental transfer. Moreover, the proportion of n-PFOS decreased significantly with the increasing concentration of Σ PFOS in the serum samples ($r=-0.342$, $p<0.001$). The mean proportion of n-PFOA in the serum samples was 99.0%, which was much higher than the technical ECF (electrochemical fluorination) products (ca. 70%). The small proportion of branched isomers of PFOA suggests that there is still a source of ECF PFOA in China. Significant correlations ($p<0.005$) were observed between the concentrations of some PFAAs with certain medical parameters in the pregnant women. For example, the levels of most perfluorinated carboxylic acids (PFCAs) were found to correlate with albumin significantly, which might be a sign of immunotoxicity of these chemicals. The adverse effects of PFAA exposure to pregnant women may increase the health risk of the fetus. Interestingly, not only the PFAA concentrations but also the percentages of PFOS and PFOA isomers were correlated with certain medical parameters. This implies that the compositions of PFOS or PFOA should be considered in human health risk assessment in the future.

Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances - a study of spouses of pregnant women in three geographical regions.
Specht I. O., Hougaard K. S., Spano M., Bizzaro D., Manicardi G. C., Lindh C. H., Toft G., Jonsson B. A., Giwercman A. and Bonde J. P.
Reprod Toxicol. 2012;33:577-83.

Perfluoroalkyl substances (PFASs) can interfere with male reproductive function, but evidence in humans is limited. Six hundred four fertile men (199 from Greenland, 197 from Poland and 208 from Ukraine) were enrolled in the study. We measured four PFASs in serum (PFOS, PFOA, PFNA and PFHxS) and concurrent DNA damage in spermatozoa by sperm chromatin structure assay (SCSA) and in situ terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, apoptotic markers in semen (Fas-receptor and Bcl-xL), and reproductive hormones in serum. No association between PFASs and SCSA, apoptotic markers or reproductive hormones emerged. We observed a slight increase in SHBG and TUNEL-positivity with increased PFOA exposure in men from Greenland. Thus, consistent evidence that PFAS

exposure interferes with sperm DNA fragmentation, apoptosis or reproductive hormones was not found.

Do perfluoroalkyl compounds impair human semen quality?
Joensen U. N., Bossi R., Leffers H., Jensen A. A., Skakkebaek N. E. and Jírgensen N. Environ Health Perspect. 2009;117:923-7.

BACKGROUND: Perfluoroalkyl acids (PFAAs) are found globally in wildlife and humans and are suspected to act as endocrine disruptors. There are no previous reports of PFAA levels in adult men from Denmark or of a possible association between semen quality and PFAA exposure.
OBJECTIVES: We investigated possible associations between PFAAs and testicular function. We hypothesized that higher PFAA levels would be associated with lower semen quality and lower testosterone levels.
METHODS: We analyzed serum samples for levels of 10 different PFAAs and reproductive hormones and assessed semen quality in 105 Danish men from the general population (median age, 19 years).
RESULTS: Considerable levels of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonic acid were found in all young men (medians of 24.5, 4.9, and 6.6 ng/mL, respectively). Men with high combined levels of PFOS and PFOA had a median of 6.2 million normal spermatozoa in their ejaculate in contrast to 15.5 million among men with low PFOS-PFOA ($p = 0.030$). In addition, we found nonsignificant trends with regard to lower sperm concentration, lower total sperm counts, and altered pituitary-gonadal hormones among men with high PFOS-PFOA levels.
CONCLUSION: High PFAA levels were associated with fewer normal sperm. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality often seen in young men. However, our findings need to be corroborated in larger studies.

D. Related studies

Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children.
Smit L. A., Lenters V., Hoyer B. B., Lindh C. H., Pedersen H. S., Liermontova I., Jonsson B. A., Piersma A. H., Bonde J. P., Toft G., Vermeulen R. and Heederik D. Allergy. 2015;70(6):653-60.

BACKGROUND: Emerging evidence suggests that prenatal or early-life exposures to environmental contaminants may contribute to an increased risk of asthma and allergies in children. We aimed to the explore associations of prenatal exposures to a large set of environmental chemical contaminants with asthma and eczema in school-age children. METHODS: We studied 1024 mother-child pairs from Greenland and Ukraine from the INUENDO birth cohort. Data were collected by means of an interview-based questionnaire when the children were 5-9 years of age. Questions from the ISAAC study were used to define asthma, eczema, and wheeze. We applied principal components analysis (PCA) to sixteen contaminants in maternal serum sampled during pregnancy, including perfluoroalkyl substances (PFASs), metabolites of diethylhexyl (DEHP) and diisononyl (DiNP) phthalates, PCB-153, and p,p'-DDE. Scores of five principal components (PCs) explaining 70% of the variance were included in multiple logistic regression models. RESULTS: In a meta-analysis that included both populations, the PC2 score, reflecting exposure to DiNP, was negatively associated with current eczema (OR 0.71, 95% CI 0.52-0.96). Other associations were not consistent between the two populations. In Ukrainian children, the PC3 score (DEHP) was positively associated with current wheeze (adjusted OR 1.56, 95% CI 1.03-2.37), whereas the PC5 score, dominated by perfluorooctanoic acid (PFOA), was inversely associated with current wheeze (OR 0.64, 0.41-0.99). In Greenlandic children, a negative association of PC4 (organochlorines) with ever eczema (OR 0.78, 0.61-0.99) was found. CONCLUSIONS: We found limited evidence to support a link between prenatal exposure to environmental chemical contaminants and childhood asthma and eczema.

Perfluoroalkyl acid contamination of follicular fluid and its consequence for in vitro oocyte developmental competence.

Petro E. M., D'Hollander W., Covaci A., Bervoets L., Fransen E., De Neubourg D., De Pauw I., Leroy J. L., Jorssen E. P. and Bols P. E.

Sci Total Environ. 2014;496:282-8.

Perfluoroalkyl acids (PFAAs) have been shown to induce negative effects in laboratory animals and in vitro experiments. Also, PFAAs have been detected in human tissues and body fluids. The ovarian follicle constitutes a fragile micro-environment where interactions between hormones, growth factors, the oocyte and surrounding somatic cells are essential to generate a fully competent oocyte. In vitro experiments suggest that PFAAs can influence this balance, but very scarce in vivo data are available to confirm this assumption. In fact, the potential PFAA-presence in the follicular micro-environment is currently unknown. Therefore, we investigated if PFAAs are present in human follicular fluid and if their presence could be a risk factor for in vivo exposed developing oocytes. Furthermore, we compared the PFAA-distribution within serum and follicular fluid. PFAAs were analyzed by LC/MS in follicular fluid (n=38) and serum (n=20) samples from women undergoing assisted reproductive technologies (ARTs). Statistical models were used to investigate PFAA-distribution in both body fluids, to compare this behavior with the distribution of lipophilic organic pollutants and to explore the relationship between patient characteristics, ART-results and follicular fluid contamination. Perfluorooctane sulfonate (PFOS) was the PFAA found in the highest concentration in follicular fluid [7.5 (0.1-30.4) ng/mL] and serum [7.6 (2.8-12.5) ng/mL]. A new variable, Principal Component 1, representing the overall PFAA-contamination of the follicular fluid samples, was associated with a higher fertilization rate ($p<0.05$) and a higher proportion of top embryos relative to the amount of retrieved oocytes ($p<0.05$), after adjusting for age, estradiol-concentration, BMI, male subfertility and the presence of other organic pollutants as explanatory variables. To conclude, overall higher PFAA-contamination in the follicular micro-environment was associated with a higher chance of an oocyte to develop into a high quality embryo. Also, PFAAs have different distribution patterns between serum and follicular fluid compared to the lipophilic organic pollutants. Further research is of course crucial to confirm these new observations.

Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood.
Okada E., Sasaki S., Kashino I., Matsuura H., Miyashita C., Kobayashi S., Itoh K., Ikeno T., Tamakoshi A. and Kishi R.
Environ Int. 2014;65:127-34.

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are detected in humans worldwide. Laboratory animal studies have shown that PFAAs are associated with immunotoxic effects. However, epidemiological studies investigating the role of PFAAs, in particular PFAAs with longer chains than perfluorooctanoic acid, are scarce. We investigated associations between prenatal exposure to PFAAs, including long-chain compounds, and infant allergic diseases at 12 and 24months in a large study population. The participants included mothers and their infants who enrolled in the Hokkaido Study on Environment and Children's Health 2003-2009. Eleven PFAAs were measured in maternal plasma taken at 28-32 weeks of gestation using ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. Characteristics of participants and information on infant allergic diseases were obtained from self-administered questionnaires and medical records. At 24 months, the adjusted odds ratio (OR) (first vs. fourth quartiles) for eczema in association with higher maternal perfluorotridecanoic acid (PFTrDA) levels was 0.62 (95% confidence interval (CI) 0.45, 0.86). After stratification by gender, the adjusted ORs in female infants from mothers with higher maternal perfluoroundecanoic acid (PFUnDA) and PFTrDA levels were also statistically significant (PFUnDA: OR=0.50; 95% CI, 0.30, 0.81; PFTrDA: OR=0.39; 95% CI, 0.23, 0.64). Our findings suggest that lower prenatal exposure to PFTrDA may decrease the risk of developing eczema in early childhood, only in female infants.

Assessment of fetal exposure and maternal elimination of perfluoroalkyl substances.
Zhang T. and Qin X.
Environ Sci Process Impacts. 2014;16(8):1878-81.

In this study, we estimated the body burden (BB) of perfluoroalkyl substances (PFASs) in a fetus at the time of delivery, and elimination of PFASs in female adults during pregnancy; and explored the isomer branching pattern-related placental transfer of perfluorooctane sulfonate (PFOS). The mean BB of PFASs were 3980 ng for PFOS and 2320 ng for perfluorooctanoic acid (PFOA), therefore, the average daily exposure

doses via placental transfer were estimated to be 13.7 and 8.32 ng per day for PFOS and PFOA, respectively, by dividing the BB of PFASs by gestational age. The total daily elimination of PFOS and PFOA in female adults through pregnancy was 30.1 and 11.4 ng per day, which indicates that pregnancy and child birth may reduce the PFASs levels in female adults. Further, branched PFOS was more readily transferred through the placenta than linear PFOS.

Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use.
Berg V., Nost T. H., Huber S., Rylander C., Hansen S., Veyhe A. S., Fuskevag O. M., Odland J. O. and Sandanger T. M.
Environ Int. 2014;69:58-66.

Determining maternal concentrations of per- and polyfluoroalkyl substances (PFASs) and the relative impact of various demographic and dietary predictors is important for assessing fetal exposure and for developing proper lifestyle advisories for pregnant women. This study was conducted to investigate maternal PFAS concentrations and their predictors in years when the production and use of several PFASs declined, and to assess the relative importance of significant predictors. Blood from 391 pregnant women participating in The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) was collected in the period 2007-2009 and serum analyses of 26 PFASs were conducted. Associations between PFAS concentrations, sampling date, and demographic and dietary variables were evaluated by multivariate analyses and linear models including relevant covariates. Parity was the strongest significant predictor for all the investigated PFASs, and nulliparous women had higher concentrations compared to multiparous women (10 ng/mL versus 4.5 ng/mL in median PFOS, respectively). Serum concentrations of PFOS and PFOA of women recruited day 1-100 were 25% and 26% higher, respectively, compared to those women recruited in the last 167 days of the study (day 601-867), and the concentrations of PFNA, PFDA and PFUnDA increased with age. Dietary predictors explained 0-17% of the variation in concentrations for the different PFASs. Significantly elevated concentrations of PFOS, PFNA, PFDA and PFUnDA were found among high consumers of marine food. The concentrations of PFHxS, PFHpS and PFNA were also increased in high consumers of game and elevated concentrations of PFHpS and PFOS were detected in high consumers of white meat. Study subjects with a high intake of salty snacks and beef had significantly higher concentrations of PFOA. The present study demonstrates that parity, sampling date and birth year are the most important predictors for maternal PFAS concentrations in years following a decrease in production and use of several

PFASs. Further, dietary predictors of PFAS concentrations were identified and varied in importance according to compound.

Perfluorinated chemicals: differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells.
Gorrochategui E., Perez-Albaladejo E., Casas J., Lacorte S. and Porte C.
Toxicol Appl Pharmacol. 2014;277(2):124-30.

The cytotoxicity of eight perfluorinated chemicals (PFCs), namely, perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorododecanoic acid (PFDoA), perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS) and perfluorooctanesulfonate (PFOS) was assessed in the human placental choriocarcinoma cell line JEG-3. Only the long chain PFCs--PFOS, PFDoA, PFNA, PFOA--showed significant cytotoxicity in JEG-3 cells with EC50 values in the range of 107 to 647 muM. The observed cytotoxicity was to some extent related to a higher uptake of the longer chain PFCs by cells (PFDoA>PFOS>>PFNA>PFOA>PFHxA). Moreover, this work evidences a high potential of PFOS, PFOA and PFBS to act as aromatase inhibitors in placental cells with IC50s in the range of 57-80 muM, the inhibitory effect of PFBS being particularly important despite the rather low uptake of the compound by cells. Finally, exposure of JEG-3 cells to a mixture of the eight PFCs (0.6 muM each) led to a relative increase (up to 3.4-fold) of several lipid classes, including phosphatidylcholines (PCs), plasmalogen PC and lyso plasmalogen PC, which suggests an interference of PFCs with membrane lipids. Overall, this work highlights the ability of the PFC mixture to alter cellular lipid pattern at concentrations well below those that generate toxicity, and the potential of the short chain PFBS, often considered a safe substitute of PFOS, to significantly inhibit aromatase activity in placental cells.

Dietary exposure to perfluoroalkyl acids of specific French adult sub-populations: high seafood consumers, high freshwater fish consumers and pregnant women.
Yamada A., Bemrah N., Veyrand B., Pollono C., Merlo M., Desvignes V., Sirot V., Marchand P., Berrebi A., Cariou R., Antignac J. P., Le Bizec B. and Leblanc J. C.
Sci Total Environ. 2014;491-492:170-5.

Perfluoroalkyl acids (PFAAs) are globally found in various media, including food and especially fishery products. In the present study, the dietary exposure to 15 perfluoroalkyl acids was assessed for 3 French adult populations, namely high seafood

consumers, high freshwater fish consumers, and pregnant women. Purified food extracts were analysed by LC-MS/MS and PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS and PFDS were monitored and quantified according to the isotope dilution principle. Under lower bound (LB) hypothesis (i.e. contamination values<LOD considered as 0), high freshwater fish consumers appear as the most exposed to PFOS (7.5 ng.kg(-1) bw.d(-1)), PFUnA (1.3 ng.kg(-1) bw.d(-1)), PFDA (0.4 ng.kg(-1) bw.d(-1)) and PFHpS (0.03 ng.kg(-1) bw.d(-1)) while high seafood consumers appear as the most exposed to PFOA (1.2 ng.kg(-1) bw.d(-1)), PFNA (0.2 ng.kg(-1) bw.d(-1)) and PFHxS (0.06 ng.kg(-1) bw.d(-1)). For all considered populations, the major exposure contributors are fish, seafood and water under LB hypothesis, while dairy products, bread and crispbread are the main contributors under upper bound (UB) hypothesis. Besides this food exposure assessment, further studies are needed to assess the more global PFAA exposure, taking into account indoor and outdoor air, dust and cutaneous contact, which could be other important contributors for this particular class of chemicals.

Polybrominated diphenyl ethers and perfluoroalkyl substances in serum of pregnant women: levels, correlations, and potential health implications.
Vorkamp K., Nielsen F., Kyhl H. B., Husby S., Nielsen L. B., Barington T., Andersson A. M. and Jensen T. K.
Arch Environ Contam Toxicol. 2014;67(1):9-20.

Polybrominated diphenyl ethers (PBDEs), a group of flame retardants, and perfluoroalkyl substances (PFASs) were analysed in serum samples of pregnant women from Denmark to provide information about their exposure and to study indications of common exposure pathways. The main BDE congener was the fully brominated BDE-209 with a median value of 7.5 ng/g lipid (46 pg/mL; 9.8 pmol/g lipid). Other BDE congeners decreased in the order BDE-47 > BDE-99 > BDE-153. The summed concentration of tri- to hepta-BDEs was 7.7 ng/g lipid, i.e. in the higher end of previously reported concentrations from Europe, including plasma samples of pregnant Danish women. Total lipid contents were relatively low, on average 5.9 g/L (9.0 mmol/L). The main PFAS compound was perfluorooctane sulfonate with a median concentration of 8.4 ng/mL. Other PFASs decreased in the order perfluorooctanoic acid > perfluorononanoic acid > perfluorodecanoic acid > perfluorohexane sulfonate and resulted in a SigmaPFAS of 12 ng/mL. Within each group, compounds were highly intercorrelated with the exception of BDE-209, which was not correlated with any of the other compounds. No correlations were found either between PFASs and PBDEs suggesting different sources of exposure and/or pharmacokinetic and metabolisation

processes. PBDE and PFAS concentrations were in the range associated with adverse effects in some epidemiological studies.

Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011.
Okada E., Kashino I., Matsuura H., Sasaki S., Miyashita C., Yamamoto J., Ikeno T., Ito Y. M., Matsumura T., Tamakoshi A. and Kishi R.
Environ Int. 2013;60:89-96.

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are used in a wide range of consumer products. Recent epidemiological studies have shown that prenatal exposure to toxic levels of PFAAs in the environment may adversely affect fetal growth and humoral immune response in infants and children. Here we have characterized levels of prenatal exposure to PFAA between 2003 and 2011 in Hokkaido, Japan, by measuring PFAA concentrations in plasma samples from pregnant women. The study population comprised 150 women who enrolled in a prospective birth cohort study conducted in Hokkaido. Eleven PFAAs were measured in maternal plasma samples using simultaneous analysis by ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. At the end of the study, in 2011, age- and parity-adjusted mean concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were 1.35ng/mL, 1.26ng/mL, 0.66ng/mL, 1.29ng/mL, 0.25ng/mL, 0.33ng/mL, 0.28ng/mL, and 3.86ng/mL, respectively. Whereas PFOS and PFOA concentrations declined 8.4%/y and 3.1%/y, respectively, PFNA and PFDA levels increased 4.7%/y and 2.4%/y, respectively, between 2003 and 2011. PFUnDA, PFDoDA, and PFTrDA were detected in the vast majority of maternal samples, but no significant temporal trend was apparent. Future studies must involve a larger population of pregnant women and their children to determine the effects of prenatal exposure to PFAA on health outcomes in infants and children.

Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population.
Borg D., Lund B. O., Lindquist N. G. and Hakansson H.
Environ Int. 2013;59:112-23.

Humans are simultaneously exposed to a multitude of chemicals. Human health risk assessment of chemicals is, however, normally performed on single substances, which

may underestimate the total risk, thus bringing a need for reliable methods to assess the risk of combined exposure to multiple chemicals. Per- and polyfluoroalkylated substances (PFASs) is a large group of chemicals that has emerged as global environmental contaminants. In the Swedish population, 17 PFASs have been measured, of which the vast majority lacks human health risk assessment information. The objective of this study was to for the first time perform a cumulative health risk assessment of the 17 PFASs measured in the Swedish population, individually and in combination, using the Hazard Index (HI) approach. Swedish biomonitoring data (blood/serum concentrations of PFASs) were used and two study populations identified: 1) the general population exposed indirectly via the environment and 2) occupationally exposed professional ski waxers. Hazard data used were publicly available toxicity data for hepatotoxicity and reproductive toxicity as well as other more sensitive toxic effects. The results showed that PFASs concentrations were in the low ng/ml serum range in the general population, reaching high ng/ml and low mug/ml serum concentrations in the occupationally exposed. For those congeners lacking toxicity data with regard to hepatotoxicity and reproductive toxicity read-across extrapolations was performed. Other effects at lower dose levels were observed for some well-studied congeners. The risk characterization showed no concern for hepatotoxicity or reproductive toxicity in the general population except in a subpopulation eating PFOS-contaminated fish, illustrating that high local exposure may be of concern. For the occupationally exposed there was concern for hepatotoxicity by PFOA and all congeners in combination as well as for reproductive toxicity by all congeners in combination, thus a need for reduced exposure was identified. Concern for immunotoxicity by PFOS and for disrupted mammary gland development by PFOA was identified in both study populations as well as a need of additional toxicological data for many PFAS congeners with respect to all assessed endpoints.

Distribution of poly- and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer.
Zhang T., Sun H., Lin Y., Qin X., Zhang Y., Geng X. and Kannan K.
Environ Sci Technol. 2013;47(14):7974-81.

Although levels of poly- and perfluoroalkyl substances (PFASs) in human maternal and neonatal blood have been widely reported in the literature, relationship of maternal-fetal transmission of PFASs with carbon chain length is presently not well understood. In this study, 11 PFASs were analyzed in matched samples, including not only maternal blood (MB, n = 31) and cord blood (CB, n = 30), but also placenta (n = 29) and amniotic fluid (AF, n = 29). Except for perfluorohexanoic acid (PFHxA), the detection frequencies of PFASs were similar among placenta, MB, and CB (>80% for 8

PFASs, nondetectable for 2 PFASs). Though only perfluorooctanoic acid (PFOA) was frequently detected (>90%) in AF, with a median concentration of 0.043 ng/mL, other 5 PFASs were also detectable in AF samples with low concentrations (mean: 0.013-0.191 ng/mL). This suggests that in addition to blood-borne in utero exposure, the fetus is also exposed to low levels of PFASs through AF. Concentrations of PFOA in AF were positively correlated with those in MB ($r = 0.738$, $p < 0.01$) and CB ($r = 0.683$, $p < 0.001$), suggesting that AF concentration could reflect fetal PFOA exposure during pregnancy and can be used as a biomarker. To clarify the effects of carbon chain length on maternal transfer of PFASs, we calculated maternal transfer efficiencies of PFASs from MB to CB (TMB-CB). A U-shaped trend in TMB-CB of C7-C12 perfluoroalkyl carboxylic acids (PFCAs) with increasing carbon chain length was found in this study for the first time. The U-shaped TMB-CB of PFCAs with carbon chain length is an integrated result of opposite trend of the ratios between MB/placenta and placenta/CB based on carbon chain length. This is the first study to report the occurrence of PFASs in human placenta. The results reported here enable better understanding of the maternal-fetal transmission of PFASs.

Neonatal-maternal factors and perfluoroalkyl substances in cord blood.
Lien G. W., Huang C. C., Wu K. Y., Chen M. H., Lin C. Y., Chen C. Y., Hsieh W. S. and Chen P. C.
Chemosphere. 2013;92(7):843-50.

Perfluoroalkyl substances (PFASs) can cross the placenta, enter fetal circulation, and were found to correlate with adverse fetal growth. However, determinants of cord blood PFASs are not fully characterized. The study aimed to explore the association between PFASs and neonatal-maternal factors within a Taiwanese birth cohort. We selected subjects from Taiwan Birth Panel Study, which enrolled 486 infant-mother pairs in 2004-2005. We collected cord blood and analyzed perfluorooctanoic acid (PFOA), perfluorooctanyl sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) using a simple protein precipitation and an ultra-high performance liquid chromatography/tandem mass spectrometry. We retrieved information pertaining to maternal socio-demographics, lifestyle- and dietary-related factors through structured questionnaires during the postpartum hospital stay. A total of 439 subjects, with 90% response rate, have completed serum analysis and questionnaire survey. The median concentrations for PFOA, PFOS, PFNA, and PFUA in cord blood were 1.86, 5.67, 3.00, and 13.5ngmL(-1), respectively. After adjusting for potential confounders, multiple linear regression models revealed that log10-PFOA was positively associated with maternal age (β=0.011) and negatively associated with multiparity (β=-0.044). Log10-PFOS was negatively correlated with birth weight (β=-

0.011) and higher maternal education (senior high school: β=-0.067; university: β=-0.088). Log10-PFUA tended to negatively associate with gender, male infants (β=-0.075), and using cosmetics during pregnancy (β=-0.065). Interestingly, presence of cockroaches in the home was positively associated with log10-PFOA (β=0.041) and 1og10-PFNA (β=0.123). In conclusion, this study demonstrated several factors to correlate with cord blood PFASs and further investigation are still needed for confirmation of exposure routes.

Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages.
Loccisano A. E., Longnecker M. P., Campbell J. L., Jr., Andersen M. E. and Clewell H. J., 3rd
J Toxicol Environ Health A. 2013;76(1):25-57.

Perfluoroalkyl acid carboxylates and sulfonates (PFAA) have many consumer and industrial applications. Developmental toxicity studies in animals have raised concern about potential reproductive/developmental effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS); however, in humans conflicting results have been reported for associations between maternal PFAA levels and these outcomes. Risk assessments and interpretation of available human data during gestation and lactation are hindered due to lack of a framework for understanding and estimating maternal, fetal, and neonatal pharmacokinetics (PK). Physiologically based pharmacokinetic (PBPK) models were developed for PFOA and PFOS for the gestation and lactation life stages in humans to understand how the physiological changes associated with development affect pharmacokinetics of these compounds in the mother, fetus, and infant. These models were derived from PBPK models for PFOA/PFOS that were previously developed for adult humans and rats during gestation and lactation and from existing human pregnancy and lactation models developed for other chemicals. The models simulated PFOA and PFOS concentrations in fetal, infant, and maternal plasma and milk, were compared to available data in humans, and also were used to estimate maternal exposure. The models reported here identified several research needs, which include (1) the identification of transporters involved in renal resorption to explain the multiyear half-lives of these compounds in humans, (2) factors affecting clearance of PFOA/PFOS during gestation and lactation, and (3) data to estimate clearance of PFOA/PFOS in infants. These models may help address concerns regarding possible adverse health effects due to PFOA/PFOS exposure in the fetus and infant and may be useful in comparing pharmacokinetics across life stages.

Effect of pregnancy on the levels of selected perfluoroalkyl compounds for females aged 17-39 years: data from National Health and Nutrition Examination Survey 2003-2008.
Jain R. B.
J Toxicol Environ Health A. 2013;76(7):409-21.

The presence of perfluoroalkyl chemicals (PFC) in maternal serum may pose a risk to the developing fetus. A large-scale study to evaluate the extent of exposure to PFC in pregnant and nonpregnant females in the United States has not been conducted. The impact of pregnancy on the concentration levels of perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorooctanoate (PFOA), and perfluorooctane sulfonate (PFOS) was assessed by analyzing data (n = 1079) from National Health and Nutrition Examination Survey (NHANES) for the years 2003-2008 for females aged 17-39 yr. While pregnant females possessed lower serum concentrations of all 4 PFC than nonpregnant females, only the differences for PFOS were significant (9.6 vs. 11.8 ng/ml). Those mothers who breast-fed at least one child displayed significantly lower levels of PFOA (2.6 vs. 3.1 ng/ml) than those with non-breast-fed infants. The concentration levels of PFNA and PFOA decreased with increase in number of live births. While levels of PFHxS and PFOS markedly fell over the period 2003-2008, the levels of PFNA rose over the same time period. There was nonlinear elevation in levels of PFHxS and PFOS with age. Smoking was associated with increased levels of PFNA and PFOA. There was a significant, positive association between total cholesterol and PFOS as well as for serum albumin with PFHxS and PFOS. Elevated levels of PFNA and PFOA were associated with a rise in serum protein. Further studies are needed to adequately explain why smoking was associated with increased levels of PFNA and PFOA.

Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds.
Ode A., Rylander L., Lindh C. H., Kallen K., Jonsson B. A., Gustafsson P., Olofsson P., Ivarsson S. A. and Rignell-Hydbom A.
Environ Sci Pollut Res Int. 2013;20(11):7970-8.

In recent years, some perfluorinated compounds (PFCs) have been identified as potentially hazardous substances which are harmful to the environment and human health. According to limited data, PFC levels in humans could be influenced by several determinants. However, the findings are inconsistent. In the present study,

perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were measured in paired maternal and cord serum samples (N=237) collected between 1978 and 2001 in Southern Sweden to study the relationship between these and to investigate several potential determinants of maternal and fetal exposure to PFCs. Time trends of PFCs in Swedish women were also evaluated. The study is a part of the Fetal Environment and Neurodevelopment Disorders in Epidemiological Research project. PFOS, PFOA, and PFNA levels (median) were higher in maternal serum (15, 2.1, and 0.24 ng/ml, respectively) than in cord serum (6.5, 1.7, and 0.20 ng/ml, respectively). PFC levels were among the highest in women originating from the Nordic countries and the lowest in women from the Middle East, North Africa, and sub-Saharan Africa. Multiparous women had lower serum PFOA levels (1.7 ng/ml) than primiparous women (2.4 ng/ml). Maternal age, body mass index, cotinine levels, and whether women carried male or female fetuses did not affect serum PFC concentrations. Umbilical cord serum PFC concentrations showed roughly similar patterns as the maternal except for the gestational age where PFC levels increased with advancing gestational age. PFOS levels increased during the study period in native Swedish women. In summary, PFOS levels tend to increase while PFOA and PFNA levels were unchanged between 1978 and 2001 in our study population. Our results demonstrate that maternal country of origin, parity, and gestational age might be associated with PFC exposure.

Placental transfer of persistent organic pollutants: a preliminary study on mother-newborn pairs.
Porpora M. G., Lucchini R., Abballe A., Ingelido A. M., Valentini S., Fuggetta E., Cardi V., Ticino A., Marra V., Fulgenzi A. R. and De Felip E.
Int J Environ Res Public Health. 2013;10(2):699-711.

The aim of this study was to characterize the placental transfer of some environmental pollutants, and to explore the possibility of quantitatively predicting in utero exposure to these contaminants from concentrations assessed in maternal blood. Levels of toxic substances such as pesticides (p,p'-DDE, β-HCH, and HCB), polychlorinated biphenyls (PCBs), perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA) were determined in serum samples of 38 pregnant women living in Rome and in samples of cord blood from their respective newborns. The study was carried out in the years 2008-2009. PCB mean concentrations in maternal serum and cord serum ranged from 0.058 to 0.30, and from 0.018 to 0.064 ng/g . fw respectively. Arithmetic means of PFOS and PFOA concentrations in mothers and newborns were 3.2 and 1.4 ng/g . fw, and 2.9 and 1.6 ng/g . fw. A strong correlation was observed between concentrations in the maternal and the foetal compartment for PFOS (Spearman r =

0.74, p < 0.001), PFOA (Spearman r = 0.70, p < 0.001), PCB 153 (Spearman r = 0.60, p < 0.001), HCB (Spearman r = 0.68, p < 0.001), PCB 180 (Spearman r = 0.55, p = 0.0012), and p,p'-DDE (Spearman r = 0.53, p = 0.0099). A weak correlation (p < 0.1) was observed for PCBs 118 and 138.

Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women.
Brantsaeter A. L., Whitworth K. W., Ydersbond T. A., Haug L. S., Haugen M., Knutsen H. K., Thomsen C., Meltzer H. M., Becher G., Sabaredzovic A., Hoppin J. A., Eggesbo M. and Longnecker M. P.
Environ Int. 2013;54:74-84.

BACKGROUND: Perfluoroalkyl substances (PFASs) are widespread pollutants that have been associated with adverse health effects although not on a consistent basis. Diet has been considered the main source of exposure. The aim of the present study was to identify determinants of four plasma PFASs in pregnant Norwegian women. METHODS: This study is based in the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health. Our sample included 487 women who enrolled in MoBa from 2003 to 2004. A questionnaire regarding sociodemographic, medical, and reproductive history was completed at 17 weeks of gestation and a dietary questionnaire was completed at 22 weeks of gestation. Maternal plasma samples were obtained around 17 weeks of gestation. Plasma concentrations of four PFASs (perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA)) were examined in relation to demographic, lifestyle, dietary, and pregnancy-related covariates. Predictors were identified by optimizing multiple linear regression models using Akaike's information criterion (AIC). RESULTS: Parity was the determinant with the largest influence on plasma PFAS concentrations, with r(2) between 0.09 and 0.32 in simple regression models. In optimal multivariate models, when compared to nulliparous women, parous women had 46%, 70%, 19%, and 62% lower concentrations of PFOS, PFOA, PFHxS, and PFNA respectively (p<0.001 except for PFHxS, p<0.01). In all these models, duration of breastfeeding was associated with reduced PFAS levels. PFOA showed the largest reduction from breastfeeding, with a 2-3% reduction per month of breastfeeding in typical cases. Levels of PFOS, PFOA, and PFNA increased with time since most recent pregnancy. While pregnancy-related factors were the most important predictors, diet was a significant factor explaining up to 4% of the variance. One quartile increase in estimated dietary PFAS intake was associated with plasma PFOS, PFOA, PFHxS, and PFNA concentration increases of 7.2%, 3.3%, 5.8% and 9.8%, respectively, resulting in small, although non-trivial

absolute changes in PFAS concentrations. CONCLUSION: Previous pregnancies and breastfeeding duration were the most important determinants of PFASs in this sample of pregnant women.

Neonatal-maternal factors and perfluoroalkyl substances in cord blood.
Lien G. W., Huang C. C., Wu K. Y., Chen M. H., Lin C. Y., Chen C. Y., Hsieh W. S. and Chen P. C.
Chemosphere. 2013;92:843-50.

Perfluoroalkyl substances (PFASs) can cross the placenta, enter fetal circulation, and were found to correlate with adverse fetal growth. However, determinants of cord blood PFASs are not fully characterized. The study aimed to explore the association between PFASs and neonatal-maternal factors within a Taiwanese birth cohort. We selected subjects from Taiwan Birth Panel Study, which enrolled 486 infant-mother pairs in 2004-2005. We collected cord blood and analyzed perfluorooctanoic acid (PFOA), perfluorooctanyl sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) using a simple protein precipitation and an ultra-high performance liquid chromatography/tandem mass spectrometry. We retrieved information pertaining to maternal socio-demographics, lifestyle- and dietary-related factors through structured questionnaires during the postpartum hospital stay. A total of 439 subjects, with 90% response rate, have completed serum analysis and questionnaire survey. The median concentrations for PFOA, PFOS, PFNA, and PFUA in cord blood were 1.86, 5.67, 3.00, and 13.5ngmL(-1), respectively. After adjusting for potential confounders, multiple linear regression models revealed that log10-PFOA was positively associated with maternal age (β=0.011) and negatively associated with multiparity (β=-0.044). Log10-PFOS was negatively correlated with birth weight (β=-0.011) and higher maternal education (senior high school: β=-0.067; university: β=-0.088). Log10-PFUA tended to negatively associate with gender, male infants (β=-0.075), and using cosmetics during pregnancy (β=-0.065). Interestingly, presence of cockroaches in the home was positively associated with log10-PFOA (β=0.041) and 1og10-PFNA (β=0.123). In conclusion, this study demonstrated several factors to correlate with cord blood PFASs and further investigation are still needed for confirmation of exposure routes.

Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan.

Hanssen L., Dudarev A. A., Huber S., Odland J. O., Nieboer E. and Sandanger T. M. Sci Total Environ. 2013;447:430-7.

Perfluoroalkyl substances (PFASs) are ubiquitous in the environment world-wide. Our overall objective was to assess the exposure to PFASs experienced by delivering women and their new-borns in the industrial city of Norilsk (arctic Russia) and the rural Aral Sea region of Uzbekistan, with the secondary objective of evaluating the distribution of PFASs between blood cell and plasma fractions. Six PFASs were detected in every sample from Norilsk city with the plasma concentration sequence of: PFOS>>PFOA>PFNA>FOSA>PFHxS>PFUnDA. In the Uzbekistani samples, only PFOS was reported above the MDL (0.08 ng/mL). The median plasma concentrations of PFOS of 11.0 ng/mL for the Norilsk mothers was comparable to that reported for western countries, while that for Uzbekistan was considerably lower (0.23 ng/mL). Apparent increases in the maternal-cord concentration ratios for both whole blood and plasma were evident with the length of the carbon chain for both the carboxylate and the sulfonate PFASs. The median value of this ratio for FOSA in plasma was the lowest, while that for whole blood was the highest. Other than for FOSA, the observed plasma-whole blood concentration ratios for maternal and umbilical cord blood were consistent with a priori calculations using appropriate packed cell and plasma volumes for neonates and pregnant women at term. Clearly FOSA favored whole blood, and acid-base equilibrium calculations suggested that the resonance-stabilized sulfonamidate ion resides in the blood cell fraction. Thus for PFASs and related compounds with pK values with magnitudes comparable to physiological pH, it is pertinent to measure the cell-associated fraction (separately or as whole blood). Our study illustrates that consideration of both the physico-chemical properties of the contaminants and the physiological attributes of blood matrices were helpful in the interpretation of our findings.

Analysis of polyfluoroalkyl substances and bisphenol A in dried blood spots by liquid chromatography tandem mass spectrometry.
Ma W., Kannan K., Wu Q., Bell E. M., Druschel C. M., Caggana M. and Aldous K. M.
Anal Bioanal Chem. 2013;405(12):4127-38.

Dried blood spots (DBS), collected as part of the newborn screening program (NSP) in the USA, is a valuable resource for studies on environmental chemical exposures and associated health outcomes in newborns. Nevertheless, determination of concentrations of environmental chemicals in DBS requires assays with great sensitivity, as the typical volume of blood available on a DBS with 16-mm diameter disc is approximately 50 muL. In this study, we developed a liquid-liquid extraction and high-performance liquid chromatography/tandem mass spectrometry method for the detection of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and bisphenol A (BPA) in DBS. The method was validated for accuracy, precision, and sensitivity, by spiking of target chemicals at different levels on Whatman 903 filter cards, which is used in the collection of DBS by the NSP. Contamination arising from collection, storage, and handling of DBS is an important issue to be considered in the analysis of trace levels of environmental chemicals in DBS. For the evaluation of the magnitude of background contamination, field blanks were prepared from unspotted portions of DBS filter cards collected by the NSP. The method was applied for the measurement of PFOS, PFOA, and BPA in 192 DBS specimens provided by NSP of New York State. PFOS and PFOA were detected in 100 % of the specimens analyzed. The concentrations of PFOS and PFOA measured in DBS were similar to those reported earlier in the whole blood samples of newborns. BPA was also found in 86 % of the specimens at concentrations ranging from 0.2 to 36 ng/mL (excluding two outliers). Further studies are needed to evaluate the sources of BPA exposures and health outcomes in newborns.

Fluorochemicals used in food packaging inhibit male sex hormone synthesis.
Rosenmai A. K., Nielsen F. K., Pedersen M., Hadrup N., Trier X., Christensen J. H. and Vinggaard A. M.
Toxicol Appl Pharmacol. 2013;266(1):132-42.

Polyfluoroalkyl phosphate surfactants (PAPS) are widely used in food contact materials (FCMs) of paper and board and have recently been detected in 57% of investigated materials. Human exposure occurs as PAPS have been measured in blood; however knowledge is lacking on the toxicology of PAPS. The aim of this study was to elucidate the effects of six fluorochemicals on sex hormone synthesis and androgen receptor (AR) activation in vitro. Four PAPS and two metabolites,

perfluorooctanoic acid (PFOA) and 8:2 fluorotelomer alcohol (8:2 FTOH) were tested. Hormone profiles, including eight steroid hormones, generally showed that 8:2 diPAPS, 8:2 monoPAPS and 8:2 FTOH led to decreases in androgens (testosterone, dehydroepiandrosterone, and androstenedione) in the H295R steroidogenesis assay. Decreases were observed for progesterone and 17-OH-progesterone as well. These observations indicated that a step prior to progestagen and androgen synthesis had been affected. Gene expression analysis of StAR, Bzrp, CYP11A, CYP17, CYP21 and CYP19 mRNA showed a decrease in Bzrp mRNA levels for 8:2 monoPAPS and 8:2 FTOH indicating interference with cholesterol transport to the inner mitochondria. Cortisol, estrone and 17β-estradiol levels were in several cases increased with exposure. In accordance with these data CYP19 gene expression increased with 8:2 diPAPS, 8:2 monoPAPS and 8:2 FTOH exposures indicating that this is a contributing factor to the decreased androgen and the increased estrogen levels. Overall, these results demonstrate that fluorochemicals present in food packaging materials and their metabolites can affect steroidogenesis through decreased Bzrp and increased CYP19 gene expression leading to lower androgen and higher estrogen levels.

Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants.

Arbuckle T. E., Kubwabo C., Walker M., Davis K., Lalonde K., Kosarac I., Wen S. W. and Arnold D. L.

Int J Hyg Environ Health. 2013;216(2):184-94.

Perfluoroalkyl acids (PFAAs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants representing two classes of environmental contaminants of toxicological concern, especially for infants. Canadian biomonitoring data on these chemicals are limited. The objectives of this study were to measure PFAAs and PBDEs in umbilical cord blood from approximately 100 hospital deliveries in Ottawa (Ontario, Canada) and examine associations with characteristics of the mother and infant. Geometric means were 1.469 ng/mL for perfluorooctanoate (PFOA) (95% confidence interval of 1.292-1.671 ng/mL), 4.443 ng/mL for perfluorooctane sulfonate (PFOS) (95% CI of 3.735-5.285 ng/mL), 0.359 ng/mL for perfluorononanoic acid (PFNA) (95% CI of 0.318-0.404 ng/mL), and 0.579 ng/mL for perfluorohexanesulfonate (PFHxS) (95% CI of 0.473-0.709 ng/mL). The final multiple regression models indicated that lower gravida, term gestational age, smoking during pregnancy and vaginal delivery were significantly associated with higher levels of PFOS. Similarly, a vaginal delivery was significantly associated with higher PFOA, while weak associations were found with lower gravida and birth weight less than 2500 g. Furthermore, higher PFNA concentrations were significantly associated with older

mothers, and vaginal delivery, while weakly associated with term gestational age. Elevated PFHxS concentrations were significantly associated with smoking during pregnancy and lower gravida. Similar to reports from other countries, the preponderant PBDE congener measured in the cord blood was PBDE-47. Questions remain on why various studies have reported conflicting results on the association between PFAAs and birth weight.

Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid.
Henry N. D. and Fair P. A.
J Appl Toxicol. 2013;33(4):265-72.

Concern with increasing levels of emerging contaminants exists on a global scale. Three commonly observed emerging environmental contaminants: triclosan (2,4,4-trichloro-2'-hydroxydiphenyl ether), a synthetic, broad-spectrum antibacterial agent, and perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), used in stain- and water-resistant treatments, have become distributed ubiquitously across ecosystems and have been detected in wildlife and humans. MCF-7 BOS human breast cancer cells were used to investigate the potential for cytotoxicity, estrogenicity and anti-estrogenicity of these three compounds at environmentally relevant concentrations using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetr azolium, inner salt assay (MTS) and the E-SCREEN bioassay. The doses used were 0.002-200 microg ml(-1) for triclosan and 0.03-30 microg ml(-1) for PFOS and PFOA. Quantitative results from the MTS assay revealed no significant cytotoxicity at lower concentrations for any of the test compounds; however, both triclosan and PFOA were cytotoxic at the highest concentrations examined (100-200 and 30 microg ml(-1), respectively), while PFOS showed no significant cytotoxicity at any of the concentrations tested. Positive estrogenic responses ($P < 0.05$) were elicited from the E-SCREEN at all concentrations examined for triclosan and PFOA and at 30 microg ml(-1) for PFOS. Further, significant anti-estrogenic activity ($P < 0.05$) was detected for all compounds tested at all concentrations when cells were co-exposed with 10(-9) m 17-β estradiol (E(2)). The overall results demonstrated that triclosan, PFOS and PFOA have estrogenic activities and that co-exposure to contaminants and E(2) produced anti-estrogenic effects. Each of these compounds could provide a source of xenoestrogens to humans and wildlife in the environment. Published 2011. This article is a US Government work and is in the public domain in the USA.

Circulating maternal perfluoroalkyl substances during pregnancy in the C8 Health Study.
Javins B., Hobbs G., Ducatman A. M., Pilkerton C., Tacker D. and Knox S. S.
Environ Sci Technol. 2013;47:1606-13.

Perfluoroalkyl substances are manmade chemicals used in many consumer products and have become ubiquitous in the environment. Animal studies and a limited number of human studies have demonstrated developmental effects in offspring exposed to perfluoroalkyl substances in utero, but the implications of timing of in utero exposure have not been systematically investigated. The present study investigated variation in perfluorocarbon levels of 9952 women of childbearing age who had been exposed to perfluorooctanoic acid (PFOA) in drinking water contaminated by industrial waste. An analysis of variance with contrast was performed to compare the levels of PFOA and perfluorooctanesulfonic acid (PFOS) in pregnant and nonpregnant women overall and during each trimester of pregnancy. We found that pregnant women had lower circulating PFOA and PFOS concentrations in peripheral blood than nonpregnant women and that PFOA levels were consistently lower throughout all trimesters for pregnancy, suggesting transfer to the fetus at an early stage of gestation. These results are discussed in the context of the endocrine-disrupting properties of perfluoroalkyl substances that have been characterized in animal and human studies. Our conclusion is that further, systematic study of the potential implications of intrauterine perfluorocarbon exposure during critical periods of fetal development is urgently needed.

Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010.
Glynn A., Berger U., Bignert A., Ullah S., Aune M., Lignell S. and Darnerud P. O.
Environ Sci Technol. 2012;46(16):9071-9.

We investigated temporal trends of blood serum levels of 13 perfluorinated alkyl acids (PFAAs) and perfluorooctane sulfonamide (FOSA) in primiparous women (N = 413) from Uppsala County, Sweden, sampled 3 weeks after delivery 1996-2010. Levels of the short-chain perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) increased 11%/y and 8.3%/y, respectively, and levels of the long-chain perfluorononanoate (PFNA) and perfluorodecanoate (PFDA) increased 4.3%/y and 3.8%/y, respectively. Concomitantly, levels of FOSA (22%/y), perfluorooctane sulfonate (PFOS, 8.4%/y), perfluorodecane sulfonate (PFDS, 10%/y), and perfluorooctanoate (PFOA, 3.1%/y) decreased. Thus, one or several sources of exposure to the latter compounds have been reduced or eliminated, whereas exposure

to the former compounds has recently increased. We explored if maternal levels of PFOS, PFOA, and PFNA during the early nursing period are representative for the fetal development period, using serial maternal serum samples, including cord blood (N = 19). PFAA levels in maternal serum sampled during pregnancy and the nursing period as well as in cord blood were strongly correlated. Strongest correlations between cord blood levels and maternal levels were observed for maternal serum sampled shortly before or after the delivery (r = 0.70-0.89 for PFOS and PFOA). A similar pattern was observed for PFNA, although the correlations were less strong due to levels close to the method detection limit in cord blood.

Changes in thyroid peroxidase activity in response to various chemicals.
Song M., Kim Y. J., Park Y. K. and Ryu J. C.
J Environ Monit. 2012;14(8):2121-6.

Thyroperoxidase (TPO) is a large heme-containing glycoprotein that catalyzes the transfer of iodine to thyroglobulin during thyroid hormone (TH) synthesis. Previously, we established an in vitro assay for TPO activity based on human recombinant TPO (hrTPO) stably transfected into human follicular thyroid carcinoma (FTC-238) cells. It is important to determine whether environmental chemicals can disrupt TPO activity because it is an important factor in the TH axis. In this study, we used our assay to examine the changes in TPO activity in response to various chemicals, including benzophenones (BPs), polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs). Overall, BPs, PAHs, and POPs slightly altered TPO activity at low doses, as compared with the positive controls methimazole (MMI), genistein, and 2,2',4,4'-tetrahydroxy BP. Benzophenone, benzhydrol, 3-methylchloranthracene, pyrene, benzo(k)fluoranthene, benzo(e)pyrene, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and heptachlor decreased TPO activity, while 2,4-dihydroxy BP, 2,2'-dihydroxy-4-methoxy BP, and dibenzo(a,h)anthracene increased TPO activity. From these data, we can predict the disruption of TPO activity by various chemicals as a sensitive TH end point. TPO activity should be considered when enacting measures to regulate environmental exposure to thyroid-disrupting chemicals.

Serum vaccine antibody concentrations in children exposed to perfluorinated compounds.

Grandjean P., Andersen E. W., Budtz-Jorgensen E., Nielsen F., Mílbak K., Weihe P. and Heilmann C.

Jama. 2012;307:391-7.

CONTEXT: Perfluorinated compounds (PFCs) have emerged as important food contaminants. They cause immune suppression in a rodent model at serum concentrations similar to those occurring in the US population, but adverse health effects of PFC exposure are poorly understood.

OBJECTIVE: To determine whether PFC exposure is associated with antibody response to childhood vaccinations.

DESIGN, SETTING, AND PARTICIPANTS: Prospective study of a birth cohort from the National Hospital in the Faroe Islands. A total of 656 consecutive singleton births were recruited during 1997-2000, [corrected] and 587 participated in follow-up through 2008.

MAIN OUTCOME MEASURES: Serum antibody concentrations against tetanus and diphtheria toxoids at ages 5 and 7 years.

RESULTS: Similar to results of prior studies in the United States, the PFCs with the highest serum concentrations were perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Among PFCs in maternal pregnancy serum, PFOS showed the strongest negative correlations with antibody concentrations at age 5 years, for which a 2-fold greater concentration of exposure was associated with a difference of -39% (95% CI, -55% to -17%) in the diphtheria antibody concentration. PFCs in the child's serum at age 5 years showed uniformly negative associations with antibody levels, especially at age 7 years, except that the tetanus antibody level following PFOS exposure was not statistically significant. In a structural equation model, a 2-fold greater concentration of major PFCs in child serum was associated with a difference of -49% (95% CI, -67% to -23%) in the overall antibody concentration. A 2-fold increase in PFOS and PFOA concentrations at age 5 years was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below a clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7 years.

CONCLUSION: Elevated exposures to PFCs were associated with reduced humoral immune response to routine childhood immunizations in children aged 5 and 7 years.

Polyfluoroalkyl compounds in Texas children from birth through 12 years of age.
Schecter A., Malik-Bass N., Calafat A. M., Kato K., Colacino J. A., Gent T. L., Hynan L. S., Harris T. R., Malla S. and Birnbaum L.
Environ Health Perspect. 2012;120(4):590-4.

BACKGROUND: For > 50 years, polyfluoroalkyl compounds (PFCs) have been used worldwide, mainly as surfactants and emulsifiers, and human exposure to some PFCs is widespread. OBJECTIVES: Our goal was to report PFC serum concentrations from a convenience sample of Dallas, Texas, children from birth to < 13 years of age, and to examine age and sex differences in PFC concentrations. METHODS: We analyzed 300 serum samples collected in 2009 for eight PFCs by online solid phase extraction-high performance liquid chromatography-isotope dilution-tandem mass spectrometry. RESULTS: Perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected in > 92% of participants; the other PFCs measured were detected less frequently. Overall median concentrations of PFOS (4.1 ng/mL) were higher than those for PFOA (2.85 ng/mL), PFNA (1.2 ng/mL), and PFHxS (1.2 ng/mL). For PFOS, PFOA, PFNA, and PFHxS, we found no significant differences ($p < 0.05$) by sex, significantly increasing concentrations for all four chemicals by age, and significantly positive correlations between all four compounds. CONCLUSIONS: We found no significant differences in the serum concentrations of PFOS, PFOA, PFNA, and PFHxS by sex, but increasing concentrations with age. Our results suggest that these 300 Texas children from birth through 12 years of age continued to be exposed to several PFCs in late 2009, years after changes in production of some PFCs in the United States.

Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia.
Fujii Y., Yan J., Harada K. H., Hitomi T., Yang H., Wang P. and Koizumi A.
Chemosphere. 2012;86(3):315-21.

In this study, 90 human breast milk samples collected from Japan, Korea, and China were analyzed for perfluorooctanoic acid (PFOA) (C8), perfluorononanoic acid (PFNA) (C9), perfluorodecanoic acid (PFDA) (C10), perfluoroundecanoic acid (PFUnDA) (C11), perfluorododecanoic acid (PFDoDA) (C12), and perfluorotridecanoic acid (PFTrDA) (C13). In addition, infant formulas (n = 9) obtained from retail stores in China and Japan were analyzed. PFOA was the predominant compound and was detected in more than 60% of samples in all three countries. The PFOA, PFNA, PFDA, and PFUnDA levels in Japan were significantly higher than those in Korea and China ($p<0.05$). The PFTrDA level was highest in Korea ($p<0.05$). The median PFOA

concentrations were 89 pg mL(-1) (48% of total perfluorinated carboxylic acids (PFCAs) (C8-C13)) in Japan, 62 pg mL(-1) (54%) in Korea, and 51 pg mL(-1) (61%) in China. The remaining Σ PFCAs (C9-C13) were 95 pg mL(-1) in Japan, 52 pg mL(-1) in Korea, and 33 pg mL(-1) in China. Among the long-chain PFCAs, odd-numbered PFCAs were more frequently detected than even-numbered PFCAs, except for PFDA in Japan. There were no evident correlations between the mother's demographic factors and the PFCA concentrations. PFOA, PFNA, and PFDA were frequently detected in both Japan and China, but there were no significant differences between the two countries. The total PFCA concentrations in the infant formulas were lower than those in the breast milk samples in Japan ($p<0.05$), but not in China ($p>0.05$). In conclusion, various PFCAs were detected in human breast milk samples from East Asian countries.

Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study.
Gutzkow K. B., Haug L. S., Thomsen C., Sabaredzovic A., Becher G. and Brunborg G. Int J Hyg Environ Health. 2012;215(2):216-9.

Perfluorinated compounds (PFCs) comprise a large group of man-made fluorinated chemicals used in a number of consumer products and industrial applications. PFCs have shown to be persistent, bio-accumulative and widespread in the environment. Animal studies have demonstrated hepatotoxicity, immunotoxicity, developmental toxicity as well as hormonal effects. We investigated prenatal exposure to several PFCs and detected up to seven different PFCs in 123 paired samples of human maternal and cord blood, from a subcohort of the Norwegian Mother and Child Cohort Study (MoBa). The maternal and foetal levels were significantly correlated for all PFCs tested with median PFC concentrations in cord blood ranging between 30 and 79% of the maternal concentrations, demonstrating placental passage. The composition of the different PFCs varied between cord and maternal blood, with a higher proportion of shorter chained PFCs together with a higher amount of the branched isomers of perfluorooctane sulfonate (PFOS) in cord blood. Additionally, the sulfonate group seems to impede transfer efficiency. This indicates a selective placental passage of the different PFCs and hence a specific foetal exposure.

Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study.
Stein C. R., Wolff M. S., Calafat A. M., Kato K. and Engel S. M.
Reprod Toxicol. 2012;34:312-6.

The extent to which polyfluoroalkyl compounds (PFCs) are detectable in amniotic fluid is unknown. Using paired samples from 28 women, we compared the concentration of 8 PFCs measured in serum, the standard matrix for assessing human exposure, amniotic fluid from routine amniocentesis, and urine. Perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS) were detected in all maternal serum samples. The number of amniotic fluid samples with detectable concentrations differed by PFC (PFOA n=24; PFNA n=10; PFOS n=9; PFHxS n=4). The correlation coefficient between maternal serum and amniotic PFC levels varied considerably by PFC (PFOA ρ =0.64, $p < 0.001$; PFNA ρ=0.05, p=0.9; PFOS ρ=0.76, p=0.01; PFHxS ρ=0.80, p=0.2). Using linear regression, PFOA appeared to be commonly detected in amniotic fluid if the serum concentration exceeded approximately 1.5 ng/mL whereas PFOS was rarely detected in amniotic fluid until the serum concentration was about 5.5 ng/mL. No PFCs were detected in urine.

Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project.
Shin H. M., Vieira V. M., Ryan P. B., Steenland K. and Bartell S. M.
Environ Health Perspect. 2011;119(12):1760-5.

BACKGROUND: People living or working in eastern Ohio and western West Virginia have been exposed to perfluorooctanoic acid (PFOA) released by DuPont Washington Works facilities. OBJECTIVES: Our objective was to estimate historical PFOA exposures and serum concentrations experienced by 45,276 non-occupationally exposed participants in the C8 Health Project who consented to share their residential histories and a 2005-2006 serum PFOA measurement. METHODS: We estimated annual PFOA exposure rates for each individual based on predicted calibrated water concentrations and predicted air concentrations using an environmental fate and transport model, individual residential histories, and maps of public water supply networks. We coupled individual exposure estimates with a one-compartment absorption, distribution, metabolism, and excretion (ADME) model to estimate time-dependent serum concentrations. RESULTS: For all participants (n = 45,276), predicted and observed median serum concentrations in 2005-2006 are 14.2 and 24.3 ppb, respectively [Spearman's rank correlation coefficient (r(s)) = 0.67]. For

participants who provided daily public well water consumption rate and who had the same residence and workplace in one of six municipal water districts for 5 years before the serum sample (n = 1,074), predicted and observed median serum concentrations in 2005-2006 are 32.2 and 40.0 ppb, respectively (r(s) = 0.82). CONCLUSIONS: Serum PFOA concentrations predicted by linked exposure and ADME models correlated well with observed 2005-2006 human serum concentrations for C8 Health Project participants. These individualized retrospective exposure and serum estimates are being used in a variety of epidemiologic studies being conducted in this region.

Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer.
Beesoon S., Webster G. M., Shoeib M., Harner T., Benskin J. P. and Martin J. W.
Environ Health Perspect. 2011;119(11):1659-64.

BACKGROUND: Perfluorochemicals (PFCs) are detectable in the general population and in the human environment, including house dust. Sources are not well characterized, but isomer patterns should enable differentiation of historical and contemporary manufacturing sources. Isomer-specific maternal-fetal transfer of PFCs has not been examined despite known developmental toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in rodents. OBJECTIVES: We elucidated relative contributions of electrochemical (phased out in 2001) and telomer (contemporary) PFCs in dust and measured how transplacental transfer efficiency (TTE; based on a comparison of maternal and cord sera concentrations) is affected by perfluorinated chain length and isomer branching pattern. METHODS: We analyzed matching samples of house dust (n = 18), maternal sera (n = 20), and umbilical cord sera (n = 20) by isomer-specific high-performance liquid chromatography tandem mass spectrometry. RESULTS: PFOA isomer signatures revealed that telomer sources accounted for 0-95% of total PFOA in house dust (median, 31%). This may partly explain why serum PFOA concentrations are not declining in some countries despite the phase-out of electrochemical PFOA. TTE data indicate that total branched isomers crossed the placenta more efficiently than did linear isomers for both PFOS ($p < 0.01$) and PFOA ($p = 0.02$) and that placental transfer of branched isomers of PFOS increased as the branching point moved closer to the sulfonate (SO3-) end of the molecule. CONCLUSIONS: Results suggest that humans are exposed to telomer PFOA, but larger studies that also account for dietary sources should be conducted. The exposure profile of PFOS and PFOA isomers can differ between the mother and fetus-an important consideration for perinatal epidemiology studies of PFCs.

Temporal changes in the levels of perfluorinated compounds in California women's serum over the past 50 years.
Wang M., Park J. S. and Petreas M.
Environ Sci Technol. 2011;45(17):7510-6.

Serum samples collected from California women at different time periods: 1960s (n = 40), 1980s (n = 30), and 2009 (n = 35) were examined for the presence of 12 perfluorinated compounds (PFCs) using an online SPE-HPLC-MS/MS method. At each time period, perfluorooctane sulfonate (PFOS) was present at the highest concentration, followed by perfluorooctanoic acid (PFOA, except in the 1960s). We found the highest levels of PFOS (median = 42.1 ng/mL) and perfluorohexane sulfonate (PFHxS, median = 1.56 ng/mL) in the 1960s samples, possibly reflecting widespread use of precursor PFCs. PFOS showed a statistically significant drop from the 1960s to the 1980s (28.8 ng/mL) and to 2009 (9.0 ng/mL), the latter being in agreement with national data. For PFOA, there was an approximately 10-fold increase in median concentrations from the 1960s (0.27 ng/mL) to the 1980s (2.71 ng/mL), and a slight drop in the 2009 samples (2.08 ng/mL). For longer chain perfluorocarboxylic acids (PFCAs), there was a continuous build-up in serum from the 1960s to 2009. To our knowledge, this is the first study to investigate temporal changes of PFCs over the past 50 years.

Comparison on gestation and lactation exposure of perfluorinated compounds for newborns.
Liu J., Li J., Liu Y., Chan H. M., Zhao Y., Cai Z. and Wu Y.
Environ Int. 2011;37(7):1206-12.

Perfluorinated compounds (PFCs) are worldwide present in the environment and the general population. Animal studies have shown developmental toxicity of these compounds. To investigate the PFCs exposure of neonates from mother during gestation and lactation, we analyzed twelve PFCs in matched maternal serum, cord serum and breast milk samples collected from 50 pairs of women and their newborns between June and July 2009 in Jinhu, China. Eight PFCs were detected in serum samples, and five of them were also detectable in breast milk. A significant intercorrelation between PFCs concentrations in matched maternal serum, cord serum and breast milk was observed ($p<0.01$, $r=0.435$-0.911). The median partition ratio was from 0.39:1 (PFDA) to 1.74:1 (PFTrDA) for seven PFCs through the placenta, and was from 0.02:1 (PFOS) to 0.09:1 (PFOA) for five PFCs through the lactation. A high transport efficiency of PFOA both through placental barrier and lactation was

observed. The postnatal exposure of PFCs through lactation was higher compared to prenatal exposure, especially for PFOA.

Analysis of perfluorinated chemicals in umbilical cord blood by ultra-high performance liquid chromatography/tandem mass spectrometry.
Lien G. W., Wen T. W., Hsieh W. S., Wu K. Y., Chen C. Y. and Chen P. C.
J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(9-10):641-6.

Perfluorinated compounds (PFCs) can cross the placental barrier and enter fetal circulation. This study aimed at developing a fast and sensitive ultra-high performance liquid chromatography/tandem mass spectrometry method for the determination of twelve perfluorinated compounds in cord blood. Samples were processed with protein precipitation using formic acid and methanol, mixed with stable isotope labeled standard, followed by sonication and centrifugation, and were analyzed using a Waters ACQUITY UPLC coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer. The instrument was operated in selected reaction monitoring (SRM) with negative electrospray ionization. Using BEH C(18) column (2.1 mmx50 mm, 1.7 mum) with 10-mM N-methylmorpholine/methanol gradient elution provided a fast chromatographic separation (5.5 min) and sharp peaks. Intra- and inter-day calibration bias was less than 7% and intra- and inter-day calibration of relative standard deviations were within 0.02-8.22% for all the analytes and concentrations. The recoveries of PFCs spiked into bovine serum ranged from 85 to 104% with relative standard deviations from 0.02 to 6.37%. The limits of quantitation (LOQs), defined as a signal-to-noise ratio of ten, ranged from 0.15 to 3.1 ng/mL for the twelve PFCs. Perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluoroundecanoic acid (PFUA) and perfluorononanoic acid (PFNA) were detected in up to 68% of umbilical cord plasma (n=444) in Taiwan Birth Panel Study and the health effect of these chemicals on children developmental deserves further investigation.

[Occurrence and relevance to health of persistent organic substances and phthalates in breast milk].
Fromme H., Raab U., Furst P., Vieth B., Volkel W., Albrecht M. and Schwegler U.
Gesundheitswesen. 2011;73(1):e27-43.

The aim of this study is to give an overview of the concentrations of persistent organic pollutants like the polychlorinated dibenzo- P-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB), polybrominated diphenyl ether (PBDE), perfluorinated compounds (PFC) and of phthalates in breast milk. On the basis of median and 95th percentile values an "average" and a "high" intake were

calculated for a 3-month-old infant exclusively breast-fed. Moreover, the actual daily intake was compared with tolerable daily intakes (TDI) recommended by scientific institutions. On this basis, we found an "average" ("high") daily intake of 70 (140) pg TEQ/kg body weight (b. w.) for PCDD/F and dioxin-like PCB (dl-PCB), 10 (20) ng/kg b. w. for PFOS (perfluorooctanesulfonate), 20 (50) ng/kg b. w. for PFOA (perfluorooctanoate), 1.7 (7.5) ng/kg b. w. for BDE 47, and 0.6 (2.1) ng/kg b. w. for BDE 99. For di-2-ethylhexyl phthalate (DEHP) and di- N-butyl phthalate (DnBP) an "average" and "high" intake of 400 ng/kg b. w. and 2,000 ng/kg b. w. and of 100 and 500 ng/kg b.w. were assumed, respectively. For all of these substances we found a daily intake via breast milk below the TDI, established on a livelong basis. On contrary, the daily intake for the sum of the PCDD/F and dl-PCB considerably exceeded the recommended TDI value. Even with regard to the "high" daily intake values the share of PBDE, PFC, and phthalates on the TDI was only in the lower percentage. Scientific organisations assume that an exceeding of the PCDD/F and dl-PCB intake in relation to the TDI value is acceptable only on the basis of the still declining levels in breast milk and the fact that this high exposure only occurs during some months of the entire life when breast milk is consumed. On the basis of the recent exposure situation mothers can exclusively breast-feed their infants for 6 months without any hesitation. The well established health benefits for mothers and infants when exclusively breast-feeding should be utilised. There is also no health concern if the mother decides to breast-feed the baby for longer than 6 months when the infant also receives additional food.

Development of an analytical strategy based on liquid chromatography-high resolution mass spectrometry for measuring perfluorinated compounds in human breast milk: application to the generation of preliminary data regarding perinatal exposure in France.
Kadar H., Veyrand B., Barbarossa A., Pagliuca G., Legrand A., Bosher C., Boquien C. Y., Durand S., Monteau F., Antignac J. P. and Le Bizec B.
Chemosphere. 2011;85(3):473-80.

Perfluorinated compounds (PFCs) are man-made chemicals for which endocrine disrupting properties and related possible side effects on human health have been reported, particularly in the case of an exposure during the early stages of development, (notably the perinatal period). Existing analytical methods dedicated to PFCs monitoring in food and/or human fluids are currently based on liquid chromatography coupled to tandem mass spectrometry, and were recently demonstrated to present some limitations in terms of sensitivity and/or specificity. An alternative strategy dedicated to the analysis of fourteen PFCs in human breast milk

was proposed, based on an effective sample preparation followed by a liquid chromatography coupled to high resolution mass spectrometry measurement (LC-HRMS). This methodology confirmed the high interest for HRMS after negative ionization for such halogenated substances, and finally permitted to reach detection limits around the pg mL(-1) range with an outstanding signal specificity compared to LC-MS/MS. The proposed method was applied to a first set of 30 breast milk samples from French women. The main PFCs detected in all these samples were PFOS and PFOA with respective median values of 74 (range from 24 to 171) and 57 (range from 18 to 102) pg mL(-1), respectively. These exposure data appeared in the same range as other reported values for European countries.

Perfluorinated chemicals in blood of residents in Wenzhou, China.
Zhang W., Lin Z., Hu M., Wang X., Lian Q., Lin K., Dong Q. and Huang C.
Ecotoxicol Environ Saf. 2011;74(6):1787-93.

Perfluorinated compounds (PFCs) are persistent organic pollutants ubiquitously distributed in the environment and human populations. Here we report PFC concentrations in the residents of Wenzhou City, which is characterized as the 'Footwear Capital' of China. Specifically, fifty serum samples collected from workers in a leather factory, fifty-five umbilical cord serum samples and fifteen serum samples from infertile men were analyzed. PFOS was one of the most frequently detected PFCs and showed the highest level. The mean serum levels of PFOS and PFOA of workers and infertile males were higher than the cord serum. PFOS concentration in cord serum increased with increase in age of the mother. Gender differences were significant both in worker serum samples and umbilical cord samples with higher levels found in males/male fetuses. Our findings suggested that PFOS, PFOA and PFHxS were widely distributed in Wenzhou residents, but occupational exposure was not the main source for workers.

A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden.
Sundstrom M., Ehresman D. J., Bignert A., Butenhoff J. L., Olsen G. W., Chang S. C. and Bergman A.
Environ Int. 2011;37(1):178-83.

The widespread presence of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexanesulfonate (PFHxS) in human general populations and their slow elimination profiles have led to renewed interest in understanding the

potential human neonatal exposures of perfluoroalkyls (PFAs) from consumption of human milk. The objective of this study was to evaluate the concentrations of PFOS, PFHxS, and PFOA in pooled human milk samples obtained in Sweden between 1972 and 2008 (a period representing the most significant period of PFA production) and to see whether the time trend of these analytes parallels that indicated in human serum. Chemical analysis of PFOS, PFHxS, and PFOA was performed on pooled Swedish human milk samples from 1972 to 2008 after methodological refinements. The 20 samples which formed the 2007 pool were also analyzed individually to evaluate sample variations. Analyses were performed by HPLC-MS/MS. Due to the complexities of the human milk matrix and the requirement to accurately quantitate low pg/mL concentrations, meticulous attention must be paid to background contamination if accurate results are to be obtained. PFOS was the predominant analyte present in the pools and all three analytes showed statistically significant increasing trends from 1972 to 2000, with concentrations reaching a plateau in the 1990s. PFOA and PFOS showed statistically significant decreasing trends during 2001-2008. At the end of the study, in 2008, the measured concentrations of PFOS, PFHxS, and PFOA in pooled human milk were 75 pg/mL, 14 pg/mL, and 74 pg/mL, respectively. The temporal concentration trends of PFOS, PFHxS, and PFOA observed in human milk are parallel to those reported in the general population serum concentrations.

Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures.
Kim S. K., Lee K. T., Kang C. S., Tao L., Kannan K., Kim K. R., Kim C. K., Lee J. S., Park P. S., Yoo Y. W., Ha J. Y., Shin Y. S. and Lee J. H.
Environ Pollut. 2011;159:169-74.

The levels of six perfluorocarboxylates (PFCAs), four perfloroalkylsulfonates (PFASs), and one sulfonamide were measured in paired samples of maternal serum, umbilical cord serum, and breast milk. The maternal and cord sera were strongly correlated with each other for all measured compounds ($r > 0.5$ and $p < 0.01$). Nevertheless, there was a significant difference in compound composition profile between the two sera matrices, with a more depletion of the longer chain compounds in cord serum. The transfer efficiency values from maternal to cord serum (TFCS/MS) decreased by 70% with each increasing unit of -CF2 chain within a PFCA group, and for perfluorooctanesulfonate (PFOS), by a half compared to perfluorooctanoate (PFOA). In contrast to the strong correlation in concentrations between the two sera matrices, the pattern of compounds in breast milk differed considerably with those in sera. Accordingly, compound- and matrix-specific transfer must be considered when assessing prenatal and postnatal exposure.

Inhibition of human and rat 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances.
Zhao B., Hu G. X., Chu Y., Jin X., Gong S., Akingbemi B. T., Zhang Z., Zirkin B. R. and Ge R. S.
Chem Biol Interact. 2010;188:38-43.

Perfluoroalkylated substances (PFASs) including perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS) have been classified as persistent organic pollutants and are known to cause reduced testosterone production in human males. The objective of the present study was to compare the potencies of five different PFASs including PFOA, PFOS, potassium perfluorooctane sulfonate (PFOSK), potassium perfluorohexane sulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in the human and rat testes. Human and rat microsomal enzymes were exposed to various PFASs. PFOS and PFOSK inhibited rat 3β-HSD activity with IC(50) of 1.35 + or -0.05 and 1.77 + or - 0.04 microM, respectively, whereas PFHxSK and PFBSK had no effect at concentrations up to 250 microM. All chemicals tested weakly inhibited human 3β-HSD activity with IC(50)s over 250 microM. On the other hand, PFOS, PFOSK and PFOA inhibited human 17β-HSD3 activity with IC(50)s of 6.02 + or - 1.02, 4.39 + or - 0.46 and 127.60 + or - 28.52 microM, respectively. The potencies for inhibition of 17β-HSD3 activity were determined to be PFOSK>PFOS>PFOA>PFHxSK=PFBSK for human 17β-HSD3 activity. There appears to be a species-dependent sensitivity to PFAS-mediated inhibition of enzyme activity because the IC(50)s of PFOS(K) for inhibition of rat 17β-HSD3 activity was greater than 250 microM. In conclusion, the present study shows that PFOS and PFOSK are potent inhibitors of rat 3β-HSD and human 17β-HSD3 activity, and implies that inhibition of steroidogenic enzyme activity may be a contributing factor to the effects that PFASs exert on androgen secretion in the testis.

Pre- and postnatal exposure to perfluorinated compounds (PFCs).
Fromme H., Mosch C., Morovitz M., Alba-Alejandre I., Boehmer S., Kiranoglu M., Faber F., Hannibal I., Genzel-Borovicz,ny O., Koletzko B. and Volkel W.
Environ Sci Technol. 2010;44:7123-9.

Perfluorinated compounds (PFCs) are a group of chemicals widely used for many applications. In this study PFCs were investigated in maternal blood during pregnancy (at two time points) (n = 40 and 38) and 6 months after delivery (n = 47), in cord blood (n = 33) and in blood of infants six (n = 40) and nineteen months (n = 24) after birth, and monthly in breast milk samples in Germany. Concentrations in maternal serum

ranged from 0.5 to 9.4 µg/L for perfluorooctane sulfonate (PFOS) and 0.7 to 8.7 µg/L for perfluorooctanoic acid (PFOA). In cord serum, the values ranged from 0.3 to 2.8 µg/L and from 0.5 to 4.2 µg/L for PFOS and PFOA, respectively. The median results from serum at six and nineteen months of age were 3.0 and 1.9 µg/L for PFOS and 6.9 and 4.6 µg/L for PFOA, respectively. In breast milk samples, PFOS ranged from < 0.03 to 0.11 µg/L (median: 0.04 µg/L), while PFOA was detected only in some samples as were all other PFCs. Overall, we found low levels of PFCs in cord sera and an increase in concentrations through the first months of infant life. Although the concentrations in breast milk were low, this intake led to a body burden at the age of six months similar to (PFOS) or higher than (PFOA) that found in adults.

Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds.

Guerrero-Preston R., Goldman L. R., Brebi-Mieville P., Ili-Gangas C., Lebron C., Witter F. R., Apelberg B. J., Hernandez-Roystacher M., Jaffe A., Halden R. U. and Sidransky D.

Epigenetics. 2010;5:539-46.

Environmental exposures in-utero may alter the epigenome, thus impacting chromosomal stability and gene expression. We hypothesized that in utero exposures to maternal smoking and perfluoroalkyl compounds (PFCs) are associated with global DNA hypomethylation in umbilical cord serum. Our objective was to determine if global DNA methylation could be used as a biomarker of in utero exposures to maternal smoking and PFCs. Using an ELISA-based method, global DNA methylation was quantified in umbilical cord serum from 30 newborns with high (> 10 ng/ml, mean 123.8 ng/ml), low (range 1-10 ng/ml, mean 1.6 ng/ml) and very low (< 1 ng/ml, mean 0.06 ng/ml) cord serum cotinine levels. Y chromosome analysis was performed to rule out maternal DNA cross-contamination. Cord serum global DNA methylation showed an inverse dose response to serum cotinine levels ($p < 0.001$). Global DNA methylation levels in cord blood were the lowest among newborns with smoking mothers (mean=15.04%; 95% CI, 8.4, 21.7) when compared to babies of mothers who were second-hand smokers (21.1%; 95% CI, 16.6, 25.5) and non-smokers (mean=29.2%; 95% CI, 20.1, 38.1). Global DNA methylation was inversely correlated with serum PFOA ($r= -0.72$, $p < 0.01$) but not PFOS levels. Serum Y chromosome analyses did not detect maternal DNA cross-contamination. This study supports the use of global DNA methylation status as a biomarker of in utero exposure to cigarette smoke and PFCs.

Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk.
Roosens L., D'Hollander W., Bervoets L., Reynders H., Van Campenhout K., Cornelis C., Van Den Heuvel R., Koppen G. and Covaci A.
Environ Pollut. 2010;158: 2546-52.

We assessed the exposure of the Flemish population to brominated flame retardants (BFRs) and perfluorinated compounds (PFCs) by analysis of pooled cord blood, adolescent and adult serum, and human milk. Levels of polybrominated diphenyl ethers (PBDEs) in blood (range 1.6-6.5 ng/g lipid weight, lw) and milk (range 2.0-6.4 ng/g lw) agreed with European data. Hexabromocyclododecane ranged between < 2.1-5.7 ng/g lw in milk. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) dominated in blood and ranged between 1 and 171 ng/mL and < 0.9-9.5 ng/mL, respectively. Total PFC levels in milk ranged between < 0.5-29 ng/mL. A significant increase in PBDE concentrations was detected from newborns (median 2.1) to the adolescents and adults (medians 3.8 and 4.6 ng/g lw, respectively). An identical trend was observed for PFOS, but not for PFOA. We estimated that newborn exposure to BFRs and PFCs occurs predominantly post-natally, whereas placental transfer has a minor impact on the body burden.

Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation.
Thomsen C., Haug L. S., Stigum H., Fríshaug M., Broadwell S. L. and Becher G.
Environ Sci Technol. 2010;44:9550-6.

At present, scientific knowledge on depuration rates of persistent organic pollutants (POPs) is limited and the previous assumptions of considerable reduction of body burdens through breast-feeding have recently been challenged. We therefore studied elimination rates of important POPs in nine Norwegian primiparous mothers and one mother breast-feeding her second child by collecting breast-milk samples (n = 70) monthly from about two weeks to up to twelve months after birth. Perfluorinated compounds (PFCs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and polychlorinated biphenyls (PCBs) were determined in the breast-milk samples. Linear mixed effect models were established for selected compounds, and significant decreases in the range of 1.2-4.7% in breast-milk concentrations per month were observed for a wide range of PCBs and PBDEs. For the first time, depuration rates for perfluorooctylsulfonate (PFOS) and perfluorooctanoic acid (PFOA) are presented, being 3.8 and 7.8% per month,

respectively ($p < 0.05$). The relative amount of the branched PFOS isomers in the breast-milk samples was 18% on average (range 6-36%, RSD 30%). There were no significant differences in isomer pattern between the mothers, or changes during the lactation period. After a year of nursing the breast-milk concentrations of PFCs, PBDEs, and PCBs were reduced by 15-94%.

Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study.
Hanssen L., Rollin H., Odland J., Oslash, Moe M. K. and Sandanger T. M.
J Environ Monit. 2010; 12: 1355-61.

There is limited information about both environmental and human perfluorinated compounds (PFCs) concentrations in the southern hemisphere, and for the first time, concentrations of these compounds are reported in maternal serum and cord blood of South African women. The majority of the participants were of African Black ethnicity, with a similar socioeconomic status. In maternal serum perfluorooctane sulfonate (PFOS) was found to be the most abundant PFC (1.6 ng mL(-1)), followed by perfluorooctanoate (PFOA: 1.3 ng mL(-1)) and perfluorohexane sulfonate (PFHxS: 0.5 ng mL(-1)); however, in cord blood PFOA was the most abundant compound (1.3 ng mL(-1)) followed by PFOS (0.7 ng mL(-1)) and PFHxS (0.3 ng mL(-1)). Linear PFOS constituted 58% of the sum of PFOS, comparable with a reported percentage from Australia. Differences in PFC concentrations between communities were found, with the highest concentrations in urban and semi-urban areas. The median maternal PFOS concentration was lower than has been reported in other studies, whereas the PFOA concentration was the same. This clearly indicates that the exposure pathway is different from the western world. Significant differences in housing quality were observed and the urban and sub-urban community had the highest living and housing standards. Possible exposure pathways could be different from those elucidated in the western world with the exception of the urban community in our study that showed higher living standards in general and easier access to modern consumer products.

Analysis of blood spots for polyfluoroalkyl chemicals.
Kato K., Wanigatunga A. A., Needham L. L. and Calafat A. M.
Anal Chim Acta. 2009;656(1-2):51-5.

Polyfluoroalkyl chemicals (PFCs) have been detected in humans, in the environment, and in ecosystems around the world. The potential for developmental and reproductive toxicities of some PFCs is of concern especially to children's health. In the United States, a sample of a baby's blood, called a "dried blood spot" (DBS), is obtained from

a heel stick within 48 h of a child's birth. DBS could be useful for assessing prenatal exposure to PFCs. We developed a method based on online solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry for measuring four PFCs in DBS, perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate, perfluorooctanoate (PFOA), and perfluorononanoate. The analytical limits of detection using one whole DBS (approximately 75 microL of blood) were <0.5 ng mL(-1). To validate the method, we analyzed 98 DBS collected in May 2007 in the United States. PFOS and PFOA were detected in all DBS at concentrations in the low ng mL(-1) range. These data suggest that DBS may be a suitable matrix for assessing perinatal exposure to PFCs, but additional information related to sampling and specimen storage is needed to demonstrate the utility of these measures for assessing exposure.

Perfluorinated compounds in delivering women from south central Vietnam.
Rylander C., Phi D. T., Odland J. O. and Sandanger T. M.
J Environ Monit. 2009;11(11):2002-8.

The associations between age, body mass index (BMI), parity, place of residence (coastal or inland) and plasma concentrations of perfluorinated compounds (PFCs) were assessed in a study population from south central Vietnam. The study group consisted of 91 delivering women of varied age (18-40 years) from two different locations (37 urban, 36 rural and 18 with unknown residence). Perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulfonate (PFHxS) were detected in 98-100% of all samples. PFOS (median 3.2 ng/ml) was the most common compound followed by PFOA (median 1.6 ng/ml), PFHxS (median 0.7 ng/ml) and perfluorononanoate (PFNA) (median 0.7 ng/ml). The women from the coastal city Nha Trang had higher concentrations of all investigated compounds than women from the inland district Dien Khanh. The two study locations are situated only 10 km apart and the diet is considered somewhat similar, however, women in Dien Khanh are more self-sufficient with locally produced food. The family income in Nha Trang is also most likely higher than in Dien Khanh and this may affect living conditions, e.g. quality of housing, which in turn may influence the exposure to PFCs. There were no associations between age, parity or BMI and the investigated PFCs. Linear PFOS constituted 83% of the sum of PFOS. This is considerably higher than reported in other studies from Europe and Australia and may indicate differences in exposure sources between countries, or differences in exposure time and persistency of the different isomers.

Polyfluoroalkyl chemicals in the serum and milk of breastfeeding women.
von Ehrenstein O. S., Fenton S. E., Kato K., Kuklenyik Z., Calafat A. M. and Hines E. P.
Reprod Toxicol. 2009;27(3-4):239-45.

Polyfluoroalkyl chemicals (PFCs) comprise a group of man-made organic compounds, some of which are persistent contaminants with developmental toxicity shown in laboratory animals. There is a paucity of human perinatal exposure data. The US EPA conducted a pilot study (Methods Advancement for Milk Analysis) including 34 breastfeeding women in North Carolina. Milk and serum samples were collected at 2-7 weeks and 3-4 months postpartum; 9 PFCs were assessed in milk and 7 in serum. Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were found in nearly 100% of the serum samples. PFOS and PFOA were found at the highest concentrations. PFCs were below the limit of quantification in most milk samples. Serum concentrations of PFOS, PFOA and PFHxS were lower ($p<0.01$) at the second visit compared to the first visit. Living in North Carolina 10 years or longer was related to elevated PFOS, PFOA and PFNA ($p<or=0.03$). These pilot data support the need to further explore perinatal PFC exposures and potentially related health effects, as planned in the upcoming National Children's Study which provided the framework for this investigation.

Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin.
Weiss J. M., Andersson P. L., Lamoree M. H., Leonards P. E., van Leeuwen S. P. and Hamers T.
Toxicol Sci. 2009;109(2):206-16.

Due to their unique surfactant properties, poly- and perfluorinated compounds (PFCs) have been extensively used and can be found all over the environment. Concern about their environmental fate and toxicological properties has initiated several research projects. In the present study, we investigated if PFCs can compete with thyroxine (T(4), i.e., the transport form of thyroid hormone) for binding to the human thyroid hormone transport protein transthyretin (TTR). Such competitive capacity may lead to decreased thyroid hormone levels as previously reported for animals exposed to PFCs. Twenty-four PFCs, together with 6 structurally similar natural fatty acids, were tested for binding capacity in a radioligand-binding assay. The binding potency decreased in the order: perfluorohexane sulfonate > perfluorooctane sulfonate/perfluorooctanoic acid > perfluoroheptanoic acid > sodium perfluoro-1-

octanesulfinate > perfluorononanoic acid, with TTR binding potencies 12.5-50 times lower than the natural ligand T(4). Some lower molecular weight compounds with structural similarity to these PFCs were > 100 times less potent than T(4). Simple descriptors based on the two-dimensional molecular structures of the compounds were used to visualize the chemical variation and to model the structure-activity relationship for the competitive potencies of the TTR-binding compounds. The models indicated the dependence on molecular size and functional groups but demanded a more detailed description of the chemical properties and data for validation and further quantitative structure-activity relationship (QSAR) development. Competitive binding of PFCs to TTR, as observed for human TTR in the present study, may explain altered thyroid hormone levels described for PFC-exposed rats and monkeys. Median human blood levels of the most potent TTR-binding PFCs are one to two orders of magnitude lower than concentration at 50% inhibition (IC(50)) values determined in the present study. In addition, this study contributes to the understanding of the bioaccumulation of PFCs in man and possibly in other wildlife species.

Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort.
Halldorsson T. I., Fei C., Olsen J., Lipworth L., McLaughlin J. K. and Olsen S. F.
Environ Sci Technol. 2008;42(23):8971-7.

This study investigated the association between dietary variables and plasma levels of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) among 1076 pregnant women. Diet was assessed at midpregnancy by a food-frequency questionnaire. Mean first trimester plasma PFOS and PFOA levels were 35.1 and 5.6 ng/mL respectively. PFOS levels were positively associated ($p < 0.05$) with intake of red meat, animal fats, and snacks (e.g., popcorn, potato chips), whereas intake of vegetables and poultry was inversely associated. The adjusted mean differences between the 75th and 25th intake percentiles were 4.3 ng/mL [95% CI: 2.1, 6.5] for red meat 3.4 ng/mL [95% CI: 1.2, 5.6] for animal fats, and 2.0 ng/mL [95% CI: 0.3, 3.6] for snacks. Similar but weaker associations were observed for PFOA. Furthermore, a comparison between women reporting low (< or =25th percentile) red meat and high (> or =75th percentile) vegetable intake and women reporting low vegetable and high red meat intake resulted in differences in plasma PFOS and PFOA concentrations equal to 31% and 18% of mean levels, respectively. Studies quantifying levels of perfluorinated compounds in food have suggested that diet could be an important route of human exposure. The observed associations in our study between dietary variables and maternal exposure further support that conclusion.

Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States.
Tao L., Ma J., Kunisue T., Libelo E. L., Tanabe S. and Kannan K.
Environ Sci Technol. 2008;42(22):8597-602.

The occurrence of perfluorinated compounds (PFCs) in human blood is known to be widespread; nevertheless, the sources of exposure to humans, including infants, are not well understood. In this study, breast milk collected from seven countries in Asia was analyzed (n=184) for nine PFCs, including perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). In addition, five brands of infant formula (n=21) and 11 brands of dairy milk (n=12) collected from retail stores in the United States were analyzed, for comparison with PFC concentrations previously reported for breast milk from the U.S. PFOS was the predominant PFC detected in almost all Asian breast milk samples, followed by perfluorohexanesulfonate (PFHxS) and PFOA. Median concentrations of PFOS in breast milk from Asian countries varied significantly;the lowest concentration of 39.4 pg/mL was found in India, and the highest concentration of 196 pg/mL was found in Japan. The measured concentrations were similarto or less than the concentrations previously reported from Sweden, the United States, and Germany (median, 106-166 pg/mL). PFHxS was found in more than 70% of the samples analyzed from Japan, Malaysia, Philippines, and Vietnam, at mean concentrations ranging from 6.45 (Malaysia) to 15.8 (Philippines) pg/mL PFOA was found frequently only in samples from Japan; the mean concentration for that country was 77.7 pg/mL. None of the PFCs were detected in the infant-formula or dairy-milk samples from the U.S. except a few samples that contained concentrations close to the limit of detection. The estimated average daily intake of PFOS by infants from seven Asian countries, via breastfeeding, was 11.8 +/- 10.6 ng/kg bw/ day; this value is 7-12 times higher than the estimated adult dietary intakes previously reported from Germany, Canada, and Spain. The average daily intake of PFOA by Japanese infants was 9.6 +/- 4.9 ng/kg bw/day, a value 3-10 times greater than the estimated adult dietary intakes reported from Germany and Canada. The highest estimated daily intakes of PFOS and PFOA by infants from seven Asian countries studied were 1-2 orders of magnitude below the tolerable daily intake values recommended by the U.K. Food Standards Agency.

Use of newborn screening program blood spots for exposure assessment: declining levels of perluorinated compounds in New York State infants.
Spliethoff H. M., Tao L., Shaver S. M., Aldous K. M., Pass K. A., Kannan K. and Eadon G. A.
Environ Sci Technol. 2008;42(14):5361-7.

Temporal biomonitoring studies can assess changes in population exposures to contaminants, but collection of biological specimens with adequate representation and sufficient temporal resolution can be resource-intensive. Newborn Screening Programs (NSPs) collect blood as dried spots on filter paper from nearly all infants born in the United States (U.S.). In this study, we investigated the use of NSP blood spots for temporal biomonitoring by analyzing perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) in 110 New York State (NYS) NSP blood spot composite specimens collected between 1997 and 2007, representing a total of 2640 infants. All analytes were detected in > or =90% of the specimens. Concentrations of PFOS, PFOSA, PFHxS, and PFOA exhibited significant exponential declines after the year 2000, coinciding with the phase-out in PFOS production in the U.S. Calculated disappearance half-lives for PFOS, PFHxS, and PFOA (4.4, 8.2, and 4.1 years, respectively) were similar to biological half-lives reported for retired fluorochemical workers. Our results suggest sharp decreases in perinatal exposure of NYS infants to PFOS, PFOSA, PFHxS, and PFOA and demonstrate, for the first time, the utility of NSP blood spots for assessment of temporal trends in exposure.

Perfluorinated compounds in human milk from Massachusetts, U.S.A.
Tao L., Kannan K., Wong C. M., Arcaro K. F. and Butenhoff J. L.
Environ Sci Technol. 2008;42(8):3096-101.

Perfluorinated compounds (PFCs), notably perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been reported in human blood. Furthermore, the occurrence of PFCs in the blood of newborn babies, coupled with the need to study the potential association of PFC exposure with birth outcomes in neonates, suggests the need for determining the sources and magnitude of exposure in infants. In this study, nine PFCs were measured in 45 human breast milk samples collected in 2004 from Massachusetts, U.S.A. PFOS and PFOA were the predominant PFCs found at mean concentrations of 131 and 43.8 pg/mL, respectively. Comparison of the ratio of PFOS to PFOA in human milk with the ratios published for human serum from the U.S. female population suggested preferential partitioning of PFOA to milk. Concentrations

of PFOA were significantly higher in the milk of mothers nursing for the first time (n = 34) than in the milk of mothers who have previously nursed (n = 8). Based on the estimated body weight and milk intake, the average and highest daily intakes of total PFCs by infants were 23.5 and 87.1 ng/kg bw, respectively. We found that the daily ingestion rates of PFOS and PFOA did not exceed the tolerable daily intake recommended by the U.K. Food Standards Agency. This is the first study to measure the occurrence of PFCs in human milk from the U.S.A.

Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland.
Apelberg B. J., Goldman L. R., Calafat A. M., Herbstman J. B., Kuklenyik Z., Heidler J., Needham L. L., Halden R. U. and Witter F. R.
Environ Sci Technol. 2007;41(11):3891-7.

Polyfluoroalkyl compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are ubiquitous, man-made chemicals. Human data suggest that in utero exposures to these chemicals occur and some evidence of developmental toxicity in animals exists. To assess the distribution and determinants of fetal exposure to PFCs, we analyzed cord serum samples from 299 singleton newborns delivered between 2004 and 2005 in Baltimore, MD for 10 PFCs by employing on-line solid-phase extraction coupled with reversed-phase high-performance liquid chromatography-tandem mass spectrometry. PFOS and PFOA were detected in 99 and 100% of umbilical cord sera, with geometric mean concentrations of 4.9 and 1.6 ng/mL, respectively. PFOS and PFOA concentrations were highly correlated (Pearson's r = 0.64 after natural log transformation, $p < 0.01$). Eight other PFCs were detected less frequently and at lower concentrations than PFOS and PFOA. Geometric mean concentrations of PFOS for Asians (6.0 ng/mL) and Blacks (5.1 ng/mL) were higher than those for Whites (4.2 ng/mL), while PFOA levels were more evenly distributed by race. Other maternal demographic and socioeconomic characteristics, including age, education, marital status, and living in the city limits were not significantly associated with cord concentrations. Our findings suggest that in utero exposure to PFOS and PFOA is ubiquitous in a population of babies born in Baltimore, MD.

Estrogenic effects of fluorotelomer alcohols for human estrogen receptor isoforms α and β in vitro.
Ishibashi H., Ishida H., Matsuoka M., Tominaga N. and Arizono K.
Biol Pharm Bull. 2007;30(7):1358-9.

The present study demonstrates the estrogenic effects of fluorotelomer alcohols (FTOHs). In a yeast two-hybrid assay, treatment with 1H,1H,2H,2H-perfluorooctan-1-ol (6:2 FTOH), 1H,1H,2H,2H-perfluoro-decan-1-ol (8:2 FTOH) and 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-1-decanol (NFDH) showed a dose-dependent interaction between the human estrogen receptor (hER) isoforms hERα or hERβ ligand-binding domain and coactivator TIF2, whereas there were no estrogenic effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) for these hERs. The estrogenic effects of FTOHs on hERα were higher than those on hERβ, indicating a differential responsiveness of hERs to FTOHs. The relative ranks of tested chemicals on the estrogenic effects for hERα and hERβ descended in the order of estradiol-17β>>>6:2 FTOH>NFDH>8:2 FTOH. These results suggest that certain FTOHs including 6:2 FTOH, 8:2 FTOH and NFDH interact with hER isoforms α and β in vitro. Further studies are necessary to investigate contamination levels, potential biological effects and the risks of these compounds on human health.

Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study.
Midasch O., Drexler H., Hart N., Beckmann M. W. and Angerer J.
Int Arch Occup Environ Health. 2007;80(7):643-8.

OBJECTIVES: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) can be released of perfluorinated compounds by biotic and/or metabolic decomposition. Due to their ubiquitous occurrence, persistence and bioaccumulative properties they can be found in blood of the general population all over the world. In animal studies PFOS and PFOA provoked cancer and showed developmental toxic potential besides other adverse health effects. On the basis of the comparison of maternal and umbilical cord plasma sample pairs we wanted to examine whether infants are exposed to PFOS and PFOA via their mothers' blood. METHODS: We determined PFOS and PFOA in 11 plasma samples of mothers and the 11 corresponding cord plasma samples of neonates. An analytical method based on plasma protein precipitation followed by HPLC with MS/MS-detection was employed. As internal standards we used 1,2,3,4-(13)C(4)-PFOS and 1,2-(13)C(2)-PFOA. RESULTS: We found PFOS and PFOA in every plasma sample analysed. In maternal plasma samples PFOS

concentrations were consistently higher compared to those of the related cord plasma samples (median: 13.0 microg/l vs. 7.3 microg/l). In the case of PFOA we observed only minor differences between PFOA concentrations within the analysed sample pairs (median: 2.6 microg/l vs. 3.4 microg/l for maternal and cord plasma samples, respectively). DISCUSSION: For both substances a crossing of the placental barrier could be shown. For PFOS we observed a decrease from maternal to cord plasma concentrations by a factor of 0.41-0.80. To the contrary, PFOA crosses the placental barrier obviously unhindered. These findings show that neonates are exposed to PFOS and PFOA via their mothers' blood. Given the current situation that only little is known about the consequences of PFOS and PFOA exposure in the early state of development of humans and the fact that in animal studies both substances showed developmental toxic effects further research regarding human health effects is indispensable.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure.
Tucker D. K., Macon M. B., Strynar M. J., Dagnino S., Andersen E. and Fenton S. E. Reprod Toxicol. 2015;54:26-36.

Perfluorooctanoic acid (PFOA) is a developmental toxicant in mice, with varied strain outcomes depending on dose and period of exposure. The impact of PFOA on female mouse pubertal development at low doses (≤1mg/kg) has yet to be determined. Therefore, female offspring from CD-1 and C57Bl/6 dams exposed to PFOA, creating serum concentrations similar to humans, were examined for pubertal onset, including mammary gland development. Pups demonstrated a shorter PFOA elimination half-life than that reported for adult mice. Prenatal exposure to PFOA caused significant mammary developmental delays in female offspring in both strains. Delays started during puberty and persisted into young adulthood; severity was dose-dependent. Also an evaluation of female serum hormone levels and pubertal timing onset revealed no effects of PFOA compared to controls in either strain. These data suggest that the mammary gland is more sensitive to early low level PFOA exposures compared to other pubertal endpoints, regardless of strain.

Hepatic Mitochondrial Alteration in CD-1 Mice Associated with Prenatal Exposures to Low Doses of Perfluorooctanoic Acid (PFOA).
Quist E. M., Filgo A. J., Cummings C. A., Kissling G. E., Hoenerhoff M. J. and Fenton S. E.
Toxicol Pathol. 2015;43(4):546-57.

Perfluorooctanoic acid (PFOA) is a perfluoroalkyl acid primarily used as an industrial surfactant. It persists in the environment and has been linked to potentially toxic and/or carcinogenic effects in animals and people. As a known activator of peroxisome proliferator-activated receptors (PPARs), PFOA exposure can induce defects in fatty acid oxidation, lipid transport, and inflammation. Here, pregnant CD-1 mice were orally gavaged with 0, 0.01, 0.1, 0.3, and 1 mg/kg of PFOA from gestation days (GD) 1 through 17. On postnatal day (PND) 21, histopathologic changes in the livers of offspring included hepatocellular hypertrophy and periportal inflammation that increased in severity by PND 91 in an apparent dose-dependent response. Transmission electron microscopy (TEM) of selected liver sections from PND 91 mice revealed PFOA-induced cellular damage and mitochondrial abnormalities with no evidence of peroxisome proliferation. Within hypertrophied hepatocytes, mitochondria were not only increased in number but also exhibited altered morphologies suggestive of increased and/or uncontrolled fission and fusion reactions. These findings suggest that peroxisome proliferation is not a component of PFOA-induced hepatic toxicity in animals that are prenatally exposed to low doses of PFOA.

In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice.
Ngo H. T., Hetland R. B., Sabaredzovic A., Haug L. S. and Steffensen I. L.
Environ Res. 2014;132:251-63.

We examined whether perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) had obesogenic effects and if they increased spontaneous intestinal tumorigenesis in the mouse model C57BL/6J-Min/+ (multiple intestinal neoplasia) after in utero exposure. The dams were exposed to PFOA or PFOS (0.01, 0.1 or 3.0mg/kg bw/day) by po gavage on GD1-17. The Min/+ and wild-type offspring were terminated at week 11 for examination of intestinal tumorigenesis or at week 20 for obesogenic effect, respectively. Body weights of the dams and pups were recorded throughout life. Food intake was determined at week 6 and 10. Blood glucose (non-fasted) was measured at week 6 and 11. No obesogenic effect of PFOA or PFOS was observed up to 20 weeks of age. PFOA or PFOS did not increase the incidence or number of

tumors in the small intestine or colon of the Min/+ mice or affect their location along the intestines. Feed intake was not affected. There were some indications of toxicity of PFOA, but not of PFOS. There was lower survival of pups after 3.0mg/kg PFOA, lower body weight in pups after 3.0 and possibly 0.1mg/kg PFOA, and increased relative liver weight after 0.01 and possibly 0.1mg/kg PFOA. Plasma glucose was lower after 0.01 and 0.1mg/kg PFOA. In conclusion, exposure to PFOA and PFOS in utero with the doses used did not have obesogenic effect on either Min/+ or wild-type mice, at least not up to 11 or 20 weeks of age, nor increased intestinal tumorigenesis in Min/+ mice.

Chronic exposure to perfluorinated compounds: Impact on airway hyperresponsiveness and inflammation.

Ryu M. H., Jha A., Ojo O. O., Mahood T. H., Basu S., Detillieux K. A., Nikoobakht N., Wong C. S., Loewen M., Becker A. B. and Halayko A. J.

Am J Physiol Lung Cell Mol Physiol. 2014;307(10):L765-74.

Emerging epidemiological evidence reveals a link between lung disease and exposure to indoor pollutants such as perfluorinated compounds (PFCs). PFC exposure during critical developmental stages may increase asthma susceptibility. Thus, in a murine model, we tested the hypothesis that early life and continued exposure to two ubiquitous household PFCs, perfluorooctanoic acid (PFOA) and perflurooctanesulfonic acid (PFOS), can induce lung dysfunction that exacerbates allergen-induced airway hyperresponsiveness (AHR) and inflammation. Balb/c mice were exposed to PFOA or PFOS (4 mg/kg chow) from gestation day 2 to 12 wk of age by feeding pregnant and nursing dams, and weaned pups. Some pups were also sensitized and challenged with ovalbumin (OVA). We assessed lung function and inflammatory cell and cytokine expression in the lung and examined bronchial goblet cell number. PFOA, but not PFOS, without the OVA sensitization/challenge induced AHR concomitant with a 25-fold increase of lung macrophages. PFOA exposure did not affect OVA-induced lung inflammatory cell number. In contrast, PFOS exposure inhibited OVA-induced lung inflammation, decreasing total cell number in lung lavage by 68.7%. Interferon-γ mRNA in the lung was elevated in all PFC-exposed groups. Despite these effects, neither PFOA nor PFOS affected OVA-induced AHR. Our data do not reveal PFOA or PFOS exposure as a risk factor for more severe allergic asthma-like symptoms, but PFOA alone can induce airway inflammation and alter airway function.

Proteomic analysis of mouse testis reveals perfluorooctanoic acid-induced reproductive dysfunction via direct disturbance of testicular steroidogenic machinery.
Zhang H., Lu Y., Luo B., Yan S., Guo X. and Dai J.
J Proteome Res. 2014;13(7):3370-85.

Perfluorooctanoic acid (PFOA) is a ubiquitous environmental pollutant suspected of being an endocrine disruptor; however, mechanisms of male reproductive disorders induced by PFOA are poorly understood. In this study, male mice were exposed to 0, 0.31, 1.25, 5, and 20 mg PFOA/kg/day by oral gavage for 28 days. PFOA significantly damaged the seminiferous tubules and reduced testosterone and progesterone levels in the testis in a dose-dependent manner. Furthermore, PFOA exposure reduced sperm quality. We identified 93 differentially expressed proteins between the control and the 5 mg/kg/d PFOA treated mice using a quantitative proteomic approach. Among them, insulin like-factor 3 (INSL3) and cytochrome P450 cholesterol side-chain cleavage enzyme (CYP11A1) as Leydig-cell-specific markers were significantly decreased. We examined in detail the expression patterns of CYP11A1 and associated genes involved in steroidogenesis in the mouse testis. PFOA inhibited the mRNA and protein levels of CYP11A1 and the mRNA levels of 17β-hydroxysteroid dehydrogenase (17β-HSD) in a dose-dependent manner. Moreover, in vitro study showed the reduction in progesterone levels was accompanied by decreased expression of CYP11A1 in cAMP-stimulated mLTC-1 cells. Our findings indicate that PFOA exposure can impair male reproductive function, possibly by disturbing testosterone levels, and CPY11A1 may be a major steroidogenic enzyme targeted by PFOA.

A species difference in the peroxisome proliferator-activated receptor α-dependent response to the developmental effects of perfluorooctanoic acid.
Albrecht P. P., Torsell N. E., Krishnan P., Ehresman D. J., Frame S. R., Chang S. C., Butenhoff J. L., Kennedy G. L., Gonzalez F. J. and Peters J. M.
Toxicological sciences : an official journal of the Society of Toxicology.
2013;131(2):568-82.

This study examined the effect of prenatal perfluorooctanoic acid (PFOA) administration on pre- and postnatal development using peroxisome proliferator-activated receptor α (PPAR α)-humanized mice to determine if species differences in receptor activity might influence the developmental effects induced by PFOA. Pregnant mice were treated daily with water or PFOA (3mg/kg) by po gavage from gestation day 1 (GD1) until GD17 and then either euthanized on GD18 or allowed to give birth and

then euthanized on postnatal day 20 (PND20). No changes in average fetal weight, crown-to-rump length, or placental weight were observed on GD18. Expression of mRNA encoding the PPAR α target genes acyl CoA oxidase (Acox1) and cytochrome P450 4a10 (Cyp4a10) in maternal and fetal liver was increased on GD18 in wild-type and PPAR α -humanized mice but not in Ppar α -null mice. On PND20, relative liver weight was higher in wild-type mice but not in Pparα-null mice or PPARα-humanized mice. Hepatic expression of Acox1 and Cyp4a10 mRNA was higher in wild-type mice but not in Pparα-null mice or PPARα-humanized mice on PND20. The percentage of mice surviving postnatally was lower in wild-type litters but not in litters from Pparα-null mice or PPARα-humanized mice. No changes in pup weight gain, onset of eye opening, or mammary gland development were found in any genotype. Results from these studies demonstrate that the developmental/postnatal effects resulting from prenatal PFOA exposure in mice are differentially mediated by mouse and human PPARα.

Neurobehavioral effects, c-Fos/Jun expression and tissue distribution in rat offspring prenatally co-exposed to MeHg and PFOA: PFOA impairs Hg retention.
Cheng J., Fujimura M., Zhao W. and Wang W.
Chemosphere. 2013;91(6):758-64.

Exposure to methylmercury (MeHg) and perfluorooctanoic acid (PFOA) can occur simultaneously as both contaminants are found in the same food sources, especially fish, seafood, marine mammals and milk. The aim of this study was to assess the effects of exposure to MeHg (10 mug mL(-1) in drinking water) and PFOA (10 mug mL(-1) in drinking water) from gestational day 1 to postnatal day (PND) 21, alone and in combination, on neurobehavioral development and the expression of c-Fos/Jun in different brain regions in the offspring. Our findings showed that exposure to MeHg alone, and exposure to MeHg combined with PFOA significantly induced cliff avoidance reflexes and negative geotaxis reflexes. And these effects appeared to be greater following exposure to MeHg alone. MeHg and/or PFOA exposure did not significantly impair motor coordination functions, or cause significant changes in c-Fos expression in the hippocampus and cerebellum, and spatial learning tests were similar to those in the controls, thus it was impossible to determine whether combined exposure to MeHg and PFOA had any additional effects on both hippocampus and cerebellum regions. However, a significant increase in the frequency of line crossing was observed in rats treated with MeHg or PFOA alone, and there were no significant differences between the MeHg+PFOA-treated group and the controls, suggesting that PFOA was antagonistic to MeHg toxicity in the locomotor activity test. Co-exposure to MeHg and PFOA decreased all tissue Hg concentrations in pups compared to the

group exposed to MeHg only, suggesting that PFOA impaired Hg retention in different tissues.

Combined effects of PFOS and PFOA on zebrafish (Danio rerio) embryos.
Ding G., Zhang J., Chen Y., Wang L., Wang M., Xiong D. and Sun Y.
Arch Environ Contam Toxicol. 2013;64(4):668-75.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two kinds of emerging contaminants most studied in recent years. However, there is limited information about their combined toxicity to aquatic organisms. In the present study, the single and combined toxicity of PFOA and PFOS to zebrafish (Danio rerio) embryos were investigated. PFOS was more toxic than PFOA for the single toxicity. In four mixtures, PFOS and PFOA showed complex interactive effects that changed from additive to synergistic effect, then to antagonistic effect, and at last turnover to synergic effect again, with increased molar ratios of PFOS. Neither the concentration-addition model nor the independent-action model could predict the combined effects when strong interactive effects existed. Although the interactive effects of PFOS and PFOA affected their combined toxicity, the trend of mixture toxicity still showed an increase with increasing molar ratios of PFOS in the mixture.

Does developmental exposure to perflurooctanoic acid (PFOA) induce immunopathologies commonly observed in neurodevelopmental disorders?
Hu Q., Franklin J. N., Bryan I., Morris E., Wood A. and DeWitt J. C.
Neurotoxicology. 2012;33(6):1491-8.

Immune comorbidities often are reported in subsets of patients with neurodevelopmental disorders, including autism spectrum disorders and attention-deficit hyperactivity disorder. A common immunopathology is an increase in serum autoantibodies against myelin basic protein (MBP) relative to control patients. Increases in autoantibodies suggest possible deficits in self-tolerance that may contribute to the formation of brain-specific autoantibodies and subsequent effects on the central nervous system (CNS). Oppositely, the formation of neuronal autoantibodies may be a reaction to neuronal injury or damage. Perfluorooctanoic acid (PFOA) is an environmental pollutant that induces multisystem toxicity in rodent models, including immunotoxicity and neurotoxicity. We hypothesized that developmental exposure to PFOA may induce immunotoxicity similar to that observed in subsets of patients with neurodevelopmental disorders. To test this hypothesis, we evaluated subsets of T cells from spleens, serum markers of autoreactivity, and levels of MBP and T cell infiltration in the cerebella of adult offspring exposed to 0.02, 0.2, or

2mg/kg of PFOA given to dams from gestation through lactation. Litter weights of offspring from dams exposed to 2mg/kg of PFOA were reduced by 32.6%, on average, from postnatal day one (PND1) through weaning (PND21). The percentage of splenic CD4+CD25+Foxp3+ T cells in male and female offspring from dams exposed to 2mg/kg of PFOA was reduced by 22% relative to the control percentage. Ex vivo co-cultures of splenic CD4+CD25+ T cells and CD4+CD25- T cells from dosed male offspring produced less IL-10 relative to control cells. Anti-ssDNA, a serum marker of autoreactivity, was decreased by 26%, on average, in female offspring from dams exposed to 0.02 and 2mg/kg PFOA. No other endpoints were statistically different by dose. These data suggest that developmental PFOA exposure may impact T cell responses and may be a possible route to downstream effects on other systems.

Perfluorooctanoic acid effects on ovaries mediate its inhibition of peripubertal mammary gland development in Balb/c and C57Bl/6 mice.
Zhao Y., Tan Y. S., Strynar M. J., Perez G., Haslam S. Z. and Yang C.
Reprod Toxicol. 2012;33(4):563-76.

Exposure to perfluorooctanoic acid (PFOA), a synthetic perfluorinated compound and an agonist of peroxisome proliferator-activated receptor α (PPARα), causes stunted mouse mammary gland development in various developmental stages. However, the underlying mechanisms remain poorly understood. We found that peripubertal PFOA exposure significantly inhibited mammary gland growth in both Balb/c and C57Bl/6 wild type mice, but not in C57Bl/6 PPARα knockout mice, and Balb/c mice were more sensitive to PFOA inhibition. PFOA caused (1) delayed or absence of vaginal opening and lack of estrous cycling during the experimental period; (2) decreases in ovarian steroid hormonal synthetic enzyme levels; and (3) reduced expression of estrogen- or progesterone-induced mammary growth factors. Supplementation with exogenous estrogen and/or progesterone reversed the PFOA inhibitory effect on mammary gland. These results indicate that PFOA effects on ovaries mediate its inhibition of mammary gland development in Balb/c and C57Bl/6 mice and that PPARα expression is a contributing factor.

Histopathologic changes in the uterus, cervix and vagina of immature CD-1 mice exposed to low doses of perfluorooctanoic acid (PFOA) in a uterotrophic assay.
Dixon D., Reed C. E., Moore A. B., Gibbs-Flournoy E. A., Hines E. P., Wallace E. A., Stanko J. P., Lu Y., Jefferson W. N., Newbold R. R. and Fenton S. E.
Reprod Toxicol. 2012;33(4):506-12.

The estrogenic and antiestrogenic potential of perfluorooctanoic acid (PFOA) was assessed using an immature mouse uterotrophic assay and by histologic evaluation of the uterus, cervix and vagina following treatment. Female offspring of CD-1 dams were weaned at 18days old and assigned to groups of equal weight, and received 0, 0.01, 0.1, or 1mg PFOA/kg BW/d by gavage with or without 17-β estradiol (E(2), 500mug/kg/d) from PND 18-20 (n=8/treatment/block). At 24h after the third dose (PND 21), uteri were removed and weighed. Absolute and relative uterine weights were significantly increased in the 0.01mg/kg PFOA only group. Characteristic estrogenic changes were present in all E(2)-treated mice; however, they were minimally visible in the 0.01 PFOA only mice. These data suggest that at a low dose PFOA produces minimal histopathologic changes in the reproductive tract of immature female mice, and does not antagonize the histopathologic effects of E(2).

Gestational and chronic low-dose PFOA exposures and mammary gland growth and differentiation in three generations of CD-1 mice.
White S. S., Stanko J. P., Kato K., Calafat A. M., Hines E. P. and Fenton S. E.
Environ Health Perspect. 2011;119(8):1070-6.

BACKGROUND: Prenatal exposure to perfluorooctanoic acid (PFOA), a ubiquitous industrial surfactant, has been reported to delay mammary gland development in female mouse offspring (F1) and the treated lactating dam (P0) after gestational treatments at 3 and 5 mg PFOA/kg/day. OBJECTIVE: We investigated the consequences of gestational and chronic PFOA exposure on F1 lactational function and subsequent development of F2 offspring. METHODS: We treated P0 dams with 0, 1, or 5 mg PFOA/kg/day on gestation days 1-17. In addition, a second group of P0 dams treated with 0 or 1 mg/kg/day during gestation and their F1 and F2 offspring received continuous PFOA exposure (5 ppb) in drinking water. Resulting adult F1 females were bred to generate F2 offspring, whose development was monitored over postnatal days (PNDs) 1-63. F1 gland function was assessed on PND10 by timed-lactation experiments. Mammary tissue was isolated from P0, F1, and F2 females throughout the study and histologically assessed for age-appropriate development. RESULTS: PFOA-exposed F1 dams exhibited diminished lactational morphology, although F1 maternal behavior and F2 offspring body weights were not significantly

affected by P0 treatment. In addition to reduced gland development in F1 females under all exposures, F2 females with chronic low-dose drinking-water exposures exhibited visibly slowed mammary gland differentiation from weaning onward. F2 females derived from 5 mg/kg PFOA-treated P0 dams displayed gland morphology similar to F2 chronic water exposure groups on PNDs 22-63. CONCLUSIONS: Gestational PFOA exposure induced delays in mammary gland development and/or lactational differentiation across three generations. Chronic, low-dose PFOA exposure in drinking water was also sufficient to alter mammary morphological development in mice, at concentrations approximating those found in contaminated human water supplies.

Prenatal perfluorooctanoic acid exposure in CD-1 mice: low-dose developmental effects and internal dosimetry.
Macon M. B., Villanueva L. R., Tatum-Gibbs K., Zehr R. D., Strynar M. J., Stanko J. P., White S. S., Helfant L. and Fenton S. E.
Toxicol Sci. 2011;122(1):134-45.

Perfluorooctanoic acid (PFOA) is an environmental contaminant that causes adverse developmental effects in laboratory animals. To investigate the low-dose effects of PFOA on offspring, timed-pregnant CD-1 mice were gavage dosed with PFOA for all or half of gestation. In the full-gestation study, mice were administered 0, 0.3, 1.0, and 3.0 mg PFOA/kg body weight (BW)/day from gestation days (GD) 1-17. In the late-gestation study, mice were administered 0, 0.01, 0.1, and 1.0 mg PFOA/kg BW/day from GD 10-17. Exposure to PFOA significantly ($p < 0.05$) increased offspring relative liver weights in all treatment groups in the full-gestation study and in the 1.0 mg PFOA/kg group in the late-gestation study. In both studies, the offspring of all PFOA-treated dams exhibited significantly stunted mammary epithelial growth as assessed by developmental scoring. At postnatal day 21, mammary glands from the 1.0 mg/kg GD 10-17 group had significantly less longitudinal epithelial growth and fewer terminal end buds compared with controls ($p < 0.05$). Evaluation of internal dosimetry in offspring revealed that PFOA concentrations remained elevated in liver and serum for up to 6 weeks and that brain concentrations were low and undetectable after 4 weeks. These data indicate that PFOA-induced effects on mammary tissue (1) occur at lower doses than effects on liver weight in CD-1 mice, an observation that may be strain specific, and (2) persist until 12 weeks of age following full-gestational exposure. Due to the low-dose sensitivity of mammary glands to PFOA in CD-1 mice, a no observable adverse effect level for mammary developmental delays was not identified in these studies.

Perfluorooctanoic acid-induced inhibition of placental prolactin-family hormone and fetal growth retardation in mice.

Suh C. H., Cho N. K., Lee C. K., Lee C. H., Kim D. H., Kim J. H., Son B. C. and Lee J. T.

Mol Cell Endocrinol. 2011;337(1-2):7-15.

Perfluorooctanoic acid (PFOA) is a persistent pollutant worldwide and even found in human cord blood and breast milk. Some animal studies have reported that PFOA causes developmental toxicity such as fetal weight loss, but the mechanism is still unclear. This study focused on developmental toxicity of PFOA, particularly impacts of PFOA on placental endocrine function such as placental prolactin (PRL)-family hormone gene expression and fetal growth in mouse. Time-mated CD-1 mice were dosed by gavage with 0, 2, 10 and 25 mg/kg B.W/day of PFOA (n-10) dissolved with de-ionized water from gestational day (GD) 11-16. During treatment, body weight of each pregnant mouse was measured daily. On day 16, caesarean sections were performed and developmental data were observed. Three placentas from three different pregnant mice were assigned to each of the following experiments. The mRNA levels of mouse placental lactogen (mPL)-II, prolactin like protein (mPLP)-E, -F and Pit-1α and β isotype mRNAs, a transacting factor of mPLs and mPLPs genes, were analyzed using northern blot, in situ hybridization and RT-PCR, respectively. Maternal body weight gain was significantly declined from GD 13 in the PFOA treated groups compared to control. Developmental data such as fetal and placental weights were significantly decreased in accordance with PFOA dosage. Number of dead fetuses and post-implantation losses were significantly increased in the PFOA-exposed groups. In addition, placental efficiency (fetal weight/placental weight) was significantly reduced in PFOA treated groups in accordance with PFOA dosage. Histopathologic changes were observed in placenta. Dose dependent necrotic changes were observed in both 10 mg and 25 mg PFOA treated groups. Cell frequency of glycogen trophoblast cell and parietal trophoblast giant cell were decreased dose dependently in the junctional zone. In the labyrinth zone, sinusoidal trophoblast giant cell frequency was decreased in the 25 mg PFOA treated group. Also, morphological change such as crushed nuclear (atrophy) of trophoblast cells was observed in 25 mg PFOA treated group. Finally, mRNA levels of the mPL-II, mPLP-E, -F and Pit-1α and β were significantly reduced in the PFOA treated groups dose dependently. In addition, the changing pattern between mPL-II, mPLP-E, -F mRNA levels and fetal body weight showed positive relationship. In conclusion, the inhibitory effects of PFOA on the placental prolactin-family hormone genes expression may be secondary effects to insufficient trophoblast cell type differentiation and/or increased trophoblast cell necrosis. The impacts of PFOA on placental development and

endocrine function reduced the placental efficiency and partly contributed to the fetal growth retardation in the mouse.

Structure-activity relationship assessment of four perfluorinated chemicals using a prolonged zebrafish early life stage test.
Hagenaars A., Vergauwen L., De Coen W. and Knapen D.
Chemosphere. 2011;82(5):764-72.

Perfluorinated compounds (PFCs) are a group of anthropogenic chemicals containing diverse functional groups and chain lengths. They are known to be persistent and bioaccumulative explaining their worldwide environmental presence. The toxicological information on these chemicals is still incomplete and insufficient to assess their environmental impact and structure-activity relationship. In the present study, the developmental effects of PFOS (perfluorooctane sulfonate, C8), PFOA (perfluorooctanoic acid, C8), PFBS (perfluorobutane sulfonate, C4) and PFBA (perfluorobutanoic acid, C4) were evaluated in zebrafish embryos (Danio rerio). The different chain lengths and functional groups of the selected chemicals made it possible to determine the structure-activity relationship of these compounds. PFCs with longer chain lengths (C8) tend to be more toxic than PFCs with shorter chain lengths (C4). Comparison based on the functional groups of compounds with the same chain length indicates that PFCs with a sulfonate group have a larger toxic potential than the ones with a carboxyl group. Furthermore, exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninflated swim bladder, as well as in more specific effects which might be related to the structure of the tested chemicals. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs. Effects on hatching rate and success were found in PFOA exposed embryos and heart rates were affected after exposure to PFOS, PFOA and PFBS.

Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner.
Onishchenko N., Fischer C., Wan Ibrahim W. N., Negri S., Spulber S., Cottica D. and Ceccatelli S.
Neurotox Res. 2011;19(3):452-61.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are organic surfactants widely used in various industrial and consumer applications. Due to their chemical properties, these perfluorinated compounds (PFCs) have also become

persistent contaminants. The risk of possible intrauterine and lactational exposure to these chemicals poses a significant health concern for potential developmental effects. In the present study we have found that dietary exposure of mice to 0.3 mg/kg of PFOS or PFOA throughout pregnancy results in different distribution pattern in the offspring brain and liver. In particular, exposure to PFOS led to four times higher accumulation of the chemical in the brains of newborn mice than PFOA. We have used a battery of behavioral tests to evaluate motor function, circadian activity, and emotion-related behavior in the exposed offspring. Exposure to PFOS resulted in decreased locomotion in a novel environment and reduced muscle strength only in male offspring. Prenatal exposure to PFOA was associated with changes in exploratory behavior in male and female offspring, as well as with increased global activity in males in their home cage. The neurobehavioral outcome of prenatal exposure to PFCs in mice is characterized by mild alterations in motor function and it appears to be sex-related.

Are developmentally exposed C57BL/6 mice insensitive to suppression of TDAR by PFOA?

Hu Q., Strynar M. J. and DeWitt J. C.
J Immunotoxicol. 2010;7(4):344-9.

Perfluorooctanoic acid (PFOA) is an environmentally persistent fluorinated compound that is present in biological samples worldwide and associated with multisystem toxicity in laboratory animal models. Several studies have reported suppression of T-cell-dependent antibody responses (TDAR) in adult rodent models after 15 or 28 days of exposure. A related compound, perfluorooctane sulfonate (PFOS), was reported to suppress TDAR in developmentally exposed mice. The developmental effects of PFOA exposure on TDAR have not been explored; therefore, the objective of our study was to determine if TDAR suppression would occur in developmentally exposed mice. Pregnant C57BL/6 mice were given 0, 0.5, or 1 mg PFOA/kg body weight (BW) in drinking water from gestation day (GD) 6 to GD17. At postnatal day (PND) 2, litters/dam were reduced to three males and three females. On PND21, female offspring were weaned and separated and on PND43, they were intravenously immunized with sheep red blood cells. Serum for evaluation of IgM titers and PFOA concentrations was collected 5 days later. Booster immunizations were given 14 days later; serum for evaluation of IgG titers and PFOA concentrations was collected 5 days later. Litter weights were statistically decreased by 10% in the 1 mg/kg group relative to controls, but liver weights, lymphoid organ weights, and TDAR did not differ in female offspring by dose. Mean PFOA serum concentrations were 122 ng/mL (0.5 mg/kg) and 183 ng/mL (1 mg/kg) and <1 ng/mL for controls. PFOA serum concentrations in offspring were 400-fold lower than serum concentrations reported to

suppress TDAR in adults; however, mice exposed during development did not survive doses higher than 1 mg/kg. Therefore, although TDAR in adult mice is sensitive to PFOA exposure, the doses and exposure scenario of this study did not induce developmental immunotoxicity (DIT). C57BL/6 mice are likely more sensitive to the overt developmental toxicity of PFOA than to potential DIT.

Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction.

Yahia D., El-Nasser M. A., Abedel-Latif M., Tsukuba C., Yoshida M., Sato I. and Tsuda S.

J Toxicol Sci. 2010;35(4):527-33.

Perfluorooctanoic acid (PFOA) has similar characteristics to perfluorooctane sulfonate (PFOS) in reproduction toxicity featured by neonatal death. We found that PFOS exposure to mice during pregnancy led to intracranial blood vessel dilatation of fetuses accompanied by severe lung collapse which caused neonatal mortality. Thus, we adopted the corresponding experimental design to PFOS in order to characterize the neonatal death by PFOA. Pregnant ICR mice were given 1, 5 and 10 mg/kg PFOA daily by gavage from gestational day (GD) 0 to 17 and 18 for prenatal and postnatal evaluations, respectively. Five to nine dams per group were sacrificed on GD 18 for prenatal evaluation; other 10 dams were left to give birth. No maternal death was observed. The liver weight increased dose-dependently, with hepatocellular hypertrophy, necrosis, increased mitosis and mild calcification at 10 mg/kg. PFOA at 10 mg/kg increased serum enzyme activities (GGT, ALT, AST and ALP) with hypoproteinemia and hypolipidemia. PFOA treatment reduced the fetal body weight at 5 and 10 mg/kg. Teratological evaluation showed delayed ossification of the sternum and phalanges and delayed eruption of incisors at 10 mg/kg, but did not show intracranial blood vessel dilatation. Postnatal evaluation revealed that PFOA reduced the neonatal survival rate at 5 and 10 mg/kg. At 5 mg/kg pups were born alive and active and 16% died within 4 days observation, while all died within 6 hr after birth at 10 mg/kg without showing intracranial blood vessel dilatation. The cause of neonatal death by PFOA may be different from PFOS.

Perfluorooctanoic acid effects on steroid hormone and growth factor levels mediate stimulation of peripubertal mammary gland development in C57BL/6 mice.
Zhao Y., Tan Y. S., Haslam S. Z. and Yang C.
Toxicol Sci. 2010;115(1):214-24.

Perfluorooctanoic acid (PFOA) is a synthetic, widely used perfluorinated carboxylic acid and a persistent environmental pollutant. It is an agonist of peroxisome proliferator-activated receptor α (PPARα). Studies have shown that PFOA causes hepatocellular hypertrophy, tumorigenesis, and developmental toxicity in rodents, and some of its toxicity depends on the expression of PPARα. Our recent study revealed a stimulatory effect of peripubertal PFOA treatment (5 mg/kg) on mammary gland development in C57Bl/6 mice. The present study was designed to examine the underlying mechanism(s). It was found that mammary gland stimulation by PFOA was similarly observed in PPARα knockout and wild-type C57Bl/6 mice. The presence of ovaries was required for PFOA treatment (5 mg/kg) to stimulate mammary gland development with significant increases in the levels of enzymes involved in steroid hormone synthesis in both PFOA-treated wild-type and PPARα knockout mouse ovaries. PFOA treatment significantly increased serum progesterone (P) levels in ovary-intact mice and also enhanced mouse mammary gland responses to exogenous estradiol (E), P, and E + P. In addition, PFOA treatment resulted in elevated mammary gland levels of epidermal growth factor receptor (EGFR), estrogen receptor α, amphiregulin (Areg, a ligand of EGFR), hepatocyte growth factor, cyclin D1, and proliferating cell nuclear antigen (PCNA) in both wild-type and PPARα knockout mouse mammary glands. These results indicate that PFOA stimulates mammary gland development in C57Bl/6 mice by promoting steroid hormone production in ovaries and increasing the levels of a number of growth factors in mammary glands, which is independent of the expression of PPARα.

[Toxicological study of PFOS/PFOA to zebrafish (Danio rerio) embryos].
Ye L., Wu L. L., Jiang Y. X., Zhang C. J. and Chen L.
Huan Jing Ke Xue. 2009;30(6):1727-32.

Acute toxicity of PFOS/PFOA to zebrafish (Danio rerio) and development effects to zebrafish embryo were examined using a zebrafish embryo test. PFOS/PFOA showed remarkably toxicity effects on zebrafish. The LC50 (48 h) values are 1 005 mg/L for PFOA, 107 mg/L for PFOS, while the LC50 (96 h) values are 499 mg/L for PFOA, 71 mg/L for PFOS. Moreover PFOS/PFOA inhibited embryo development, and caused embryo abnormality and death. After exposure to high concentration of PFOS (> 240

mg/L), cells in animal pole of embryos autolyzed to coagulate, which indicated PFOS caused cell membranes damage. The most sensitive endpoints for PFOS exposure is spinal column malformation, and the EC50 values is 9.14 mg/L. While for PFOA hatching (96 h) is the most sensitive, and the EC50 values is 328.0 mg/L. Both PFOS and PFOA retarded embryo development which indicates their development toxicity.

B. Studies reporting no developmental or reproductive toxicity

Comparative in vivo and in vitro analysis of possible estrogenic effects of perfluorooctanoic acid.
Yao P. L., Ehresman D. J., Rae J. M., Chang S. C., Frame S. R., Butenhoff J. L., Kennedy G. L. and Peters J. M.
Toxicology. 2014;326:62-73.

Previous studies suggested that perfluorooctanoate (PFOA) could activate the estrogen receptor (ER). The present study examined the hypothesis that PFOA can activate ER using an in vivo uterotrophic assay in CD-1 mice and an in vitro reporter assay. Pre-pubertal female CD-1 mice fed an estrogen-free diet from postnatal day (PND)14 through weaning on PND18 were administered 0, 0.005, 0.01, 0.02, 0.05, 0.1, or 1mg/kg PFOA or 17β-estradiol (E2, 0.5mg/kg) from PND18-20. In contrast to E2, PFOA caused no changes in the relative uterine weight, the expression of ER target genes, or the morphology of the uterus/cervix and/or vagina on PND21. Treatment of a stable human cell line containing an ER-dependent luciferase reporter construct with a broad concentration range of PFOA caused no change in ER-dependent luciferase activity; whereas E2 caused a marked increase of ER-dependent luciferase activity. These data indicate that PFOA does not activate mouse or human ER.

Male reproductive system parameters in a two-generation reproduction study of ammonium perfluorooctanoate in rats and human relevance.
York R. G., Kennedy G. L., Jr., Olsen G. W. and Butenhoff J. L.
Toxicology. 2010;271(1-2):64-72.

Ammonium perfluorooctanoate (ammonium PFOA) is an industrial surfactant that has been used primarily as a processing aid in the manufacture of fluoropolymers. The environmental and metabolic stability of PFOA together with its presence in human blood and long elimination half-life have led to extensive toxicological studies in laboratory animals. Two recent publications based on observations from the Danish

general population have reported: (1) a negative association between serum concentrations of PFOA in young adult males and their sperm counts and (2) a positive association among women with time to pregnancy. A two-generation reproduction study in rats was previously published (2004) in which no effects on functional reproduction were observed at doses up to 30mg ammonium PFOA/kg body weight. The article contained the simple statement: "In males, fertility was normal as were all sperm parameters". In order to place the recent human epidemiological data in perspective, herein we provide the detailed male reproductive parameters from that study, including sperm quality and testicular histopathology. Sperm parameters in rats from the two-generation study in all ammonium PFOA treatment groups were unaffected by treatment with ammonium PFOA. These observations reflected the normal fertility observations in these males. No evidence of altered testicular and sperm structure and function was observed in ammonium PFOA-treated rats whose mean group serum PFOA concentrations ranged up to approximately 50,000ng/mL. Given that median serum PFOA in the Danish cohorts was approximately 5ng/mL, it seems unlikely that concentrations observed in the general population, including those recently reported in Danish general population, could be associated causally with a real decrement in sperm number and quality.

C. Related articles

Evolutionary ecotoxicology of perfluoralkyl substances (PFASs) inferred from multigenerational exposure: a case study with Chironomus riparius (Diptera, Chironomidae).
Stefani F., Rusconi M., Valsecchi S. and Marziali L.
Aquat Toxicol. 2014;156:41-51.

A multigeneration toxicity test on Chironomus riparius was performed with the aim of investigating the evolutionary consequences of exposure to perfluoralkyl substances (perfluorooctane sulfonic acid, PFOS; perfluorooctanoic acid, PFOA; perfluorobutane sulfonate, PFBS). Six-hundred larvae were bred per treatment and per generation until emergence and egg deposition under a nominal concentration of 10mug/L of contaminants. Newborn larvae were used to start the next generation. Evolution of genetic variability was evaluated along a total of 10 consecutive generations based on 5 microsatellite loci. Analysis of life-history traits (survival, sex ratio and reproduction) was also carried out. Rapid genetic variability reduction was observed in all treatments, including controls, across generations due to the test conditions. Nevertheless, an increased mutation rate determined a stronger conservation of genetic variability in

PFOS and, at minor extent, in PFBS exposed populations compared to controls. No significant effects were induced by exposure to PFOA. Direct mutagenicity or induced stress conditions may be at the base of increased mutation rate, indicating the potential risk of mutational load caused by exposure to PFOS and PFBS. The test provided the opportunity to evaluate the use of approximate Bayesian computation (ABC) and coalescent approaches in evolutionary ecotoxicology. A weak performance was evidenced for ABC, either in terms of bias or dispersion of effective population sizes and of estimates of mutation rate. On the contrary, coalescent simulations proved the sensitivity of traditional genetic endpoints (i.e. heterozygosity and number of alleles) to the alteration of mutation rate, but not to erosion of genetic effective size.

Chronic effects of PFOA and PFOS on sexual reproduction of freshwater rotifer Brachionus calyciflorus.
Zhang L., Niu J., Wang Y., Shi J. and Huang Q.
Chemosphere. 2014;114:114-20.

Rotifers play an important role in the dynamics of freshwater and coastal marine ecosystems, and are also important tools for assessing toxicity in aquatic environments. In this study, the effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on the population growth rate and resting egg production of rotifer Brachionus calyciflorus were investigated. Reproductive bioassays indicated that PFOS increased the rotifer population growth rate at the concentration 2.0 mg L(-1), and inhibited it at higher concentrations. For PFOA, the inhibition of population growth rate was observed when the concentration was greater than 4.0 mg L(-1). Exposure to PFOS (0.25 mg L(-1)) or PFOA (2.0 mg L(-1)) increased the mictic ratios of unexposed rotifer offspring. Population variation and increased mictic ratios were likely the two major factors leading to decline of resting egg production. The resting eggs formed under exposure to PFOA/PFOS in the range of 0.125-2.0 mg L(-1) showed higher hatching percentages in the control medium than that without PFOA/PFOS exposure. When the resting eggs were formed in the control medium and incubated in media with different levels of PFOA/PFOS, higher hatching percentages were induced by PFOS but lower hatching percentages induced by PFOA. The effects on the hatching rate of resting eggs with PFOA/PFOS exposure during the hatching period were greater than those with exposure during resting egg formation period, and the effect of PFOS was greater than that of PFOA. Both PFOA and PFOS exhibited slight effect on the hatching pattern.

Endocrine disruptors differentially target ATP-binding cassette transporters in the blood-testis barrier and affect Leydig cell testosterone secretion in vitro.
Dankers A. C., Roelofs M. J., Piersma A. H., Sweep F. C., Russel F. G., van den Berg M., van Duursen M. B. and Masereeuw R.
Toxicol Sci. 2013;136(2):382-91.

Endocrine-disrupting chemicals (EDCs) are considered to cause testicular toxicity primarily via interference with steroid hormone function. Alternatively, EDCs could possibly exert their effects by interaction with ATP-binding cassette (ABC) transporters that are expressed in the blood-testis barrier. In this study, we investigated the effects of bisphenol A (BPA), tetrabromobisphenol A (TBBPA), bis(2-ethylhexyl) phthalate, mono(2-ethylhexyl) phthalate, perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS) on breast cancer resistance protein (BCRP), multidrug resistance proteins 1 and 4 (MRP1 and MRP4), and P-glycoprotein (P-gp) using membrane vesicles overexpressing these transporters. BPA solely inhibited BCRP activity, whereas TBBPA, PFOA, and PFOS inhibited all transporters tested. No effect was observed for the phthalates. Using transporter-overexpressing Madin-Darby canine kidney cells, we show that BPA and PFOA, but not TBBPA, are transported by BCRP, whereas none of the compounds were transported by P-gp. To investigate the toxicological implications of these findings, testosterone secretion and expression of steroidogenic genes were determined in murine Leydig (MA-10) cells upon exposure to the selected EDCs. Only BPA and TBBPA concentration dependently increased testosterone secretion by MA-10 cells to 6- and 46-fold of control levels, respectively. Inhibition of the Mrp's by MK-571 completely blocked testosterone secretion elicited by TBBPA, which could not be explained by coinciding changes in expression of steroidogenic genes. Therefore, we hypothesize that transporter-mediated efflux of testosterone precursors out of MA-10 cells is inhibited by TBBPA resulting in higher availability for testosterone production. Our data show the toxicological and clinical relevance of ABC transporters in EDC risk assessment related to testicular toxicity.

Perfluorooctanoate suppresses spheroid attachment on endometrial epithelial cells through peroxisome proliferator-activated receptor α and down-regulation of Wnt signaling.
Tsang H., Cheung T. Y., Kodithuwakku S. P., Chai J., Yeung W. S., Wong C. K. and Lee K. F.
Reprod Toxicol. 2013;42:164-71.

Exposure of animals to perfluorooctanoic acid (PFOA), a surfactant used in emulsion polymerization processes causes early pregnancy loss, delayed growth and

development of fetuses. The mechanisms of action are largely unknown. We studied the effect of PFOA on implantation using an in vitro spheroid-endometrial cell co-culture model. PFOA (10-100muM) significantly reduced Jeg-3 spheroid attachment on RL95-2 endometrial cells. PFOA also suppressed β-catenin expression in Jeg-3 cells. The Wnt agonist Wnt3a stimulated β-catenin expression in Jeg-3 cells and reversed the PFOA suppression of the spheroid attachment. The putative PFOA receptors (PPARα, β, γ) present in both cell lines were not affected by PFOA (0.01-100muM). The PPARα antagonist MK886 restored the β-catenin and E-cadherin expression levels in Jeg-3 cells and reversed the suppression of the spheroid attachment caused by PFOA. Taken together, PFOA suppresses spheroid attachment through PPARα and Wnt signaling pathways via down-regulation of β-catenin and E-cadherin expression.

Cytotoxicity and inhibition of intercellular interaction in N2a neurospheroids by perfluorooctanoic acid and perfluorooctanesulfonic acid.
Choi S. K., Kim J. H., Park J. K., Lee K. M., Kim E. and Jeon W. B.
Food Chem Toxicol. 2013;60:520-9.

Effects of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) on the neuronal lineage marker expression, cell-cell interaction, caspase-3 mRNA transcription and reactive oxygen species production by N2a neuronal cells were assesses in 3-dimensional (3D) spheroid cultures, and the cytotoxicity were thoroughly compared with those of a conventional 2D monolayer-based toxicity assay. Increasing concentrations of PFOA or PFOS resulted in an increase in cell death. The half maximal inhibitory concentrations measured with spheroids were approximately one and a half times greater than the respective values for monolayer cells. Necrosis was prevalent in spheroids regardless of the dose, whereas the major injury mechanism in monolayers was dependent on compound concentration. Both PFOA and PFOS inhibited neuronal, astrocyte and oligodendrocyte marker gene expression by monolayers and spheroids grown under undifferentiated and all-trans-retinoic acid-induced differentiating conditions. In the presence of PFOA or PFOS, expression levels of E-cadherin and connexin-43 mRNAs were significantly downregulated, and spheroids were dissociated into single cell populations, indicating that the compounds affect the synthesis of E-cadherin and connexin-43 at the transcriptional level. Results from 3D cultures may provide an insight into potential inhibitory mode of action on gap junctional intercellular communication.

Perfluorooctanoic acid induced-developmental cardiotoxicity: are peroxisome proliferator activated receptor α (PPARα) and bone morphorgenic protein 2 (BMP2) pathways involved?
Jiang Q., Lust R. M. and DeWitt J. C.
J Toxicol Environ Health A. 2013;76(11):635-50.

Perfluorooctanoic acid (PFOA) is an environmental contaminant known to induce developmental toxicity in animal models through activation of the peroxisome proliferator-activated receptor α (PPARα). Previously, it was demonstrated that in ovo exposure to PFOA induced cardiotoxicity in chicken embryos and hatchlings. To investigate potential PPARα-mediated mechanisms, fertile chicken eggs were injected prior to incubation with WY 14,643, a PPARα agonist. Cardiac morphology and function were evaluated in late-stage embryos and hatchlings. Histologically, unlike PFOA, WY 14,643 did not induce thinning of the right ventricular wall. Via echocardiography, however, WY 14,643 induced effects similar to those of PFOA, including increased left ventricular wall thickness and mass, elevated heart rate, ejection fraction, fractional shortening, and decreased stroke volume. Additionally, to investigate mechanisms associated with early heart development, a separate group of fertile chicken eggs was injected prior to incubation with PFOA or WY 14,643 and in early-stage embryos, gene expression and protein concentration associated with the bone morphogenic protein (BMP2) pathway were determined. Although changes were not statistically consistent among doses, expression of BMP2, Nkx2.5, and GATA4 mRNA in early embryos was altered by PFOA exposure; however, protein concentrations of these targets were not markedly altered by either PFOA or WY 14,643. Protein levels of pSMAD1/5, a transcriptional regulator stimulated by BMPs, were altered by both PFOA and WY 14,643, but in different directions; PFOA reduced cytoplasmic pSMAD1/5, whereas WY 14,643 decreased nuclear pSMAD1/5. Taken together, these data suggest that developmental cardiotoxicity induced by PFOA likely involves both PPARα and BMP2 pathways.

In vivo evaluation and comparison of developmental toxicity and teratogenicity of perfluoroalkyl compounds using Xenopus embryos.
Kim M., Son J., Park M. S., Ji Y., Chae S., Jun C., Bae J. S., Kwon T. K., Choo Y. S., Yoon H., Yoon D., Ryoo J., Kim S. H., Park M. J. and Lee H. S.
Chemosphere. 2013;93(6):1153-60.

Perfluoroalkyl compounds (PFCs) are environmental toxicants that persistently accumulate in human blood. Their widespread detection and accumulation in the environment raise concerns about whether these chemicals might be developmental

toxicants and teratogens in ecosystem. We evaluated and compared the toxicity of PFCs of containing various numbers of carbon atoms (C8-11 carbons) on vertebrate embryogenesis. We assessed the developmental toxicity and teratogenicity of various PFCs. The toxic effects on Xenopus embryos were evaluated using different methods. We measured teratogenic indices (TIs), and investigated the mechanisms underlying developmental toxicity and teratogenicity by measuring the expression of organ-specific biomarkers such as xPTB (liver), Nkx2.5 (heart), and Cyl18 (intestine). All PFCs that we tested were found to be developmental toxicants and teratogens. Their toxic effects were strengthened with increasing length of the fluorinated carbon chain. Furthermore, we produced evidence showing that perfluorodecanoic acid (PFDA) and perfluoroundecanoic acid (PFuDA) are more potent developmental toxicants and teratogens in an animal model compared to the other PFCs we evaluated [perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA)]. In particular, severe defects resulting from PFDA and PFuDA exposure were observed in the liver and heart, respectively, using whole mount in situ hybridization, real-time PCR, pathologic analysis of the heart, and dissection of the liver. Our studies suggest that most PFCs are developmental toxicants and teratogens, however, compounds that have higher numbers of carbons (i.e., PFDA and PFuDA) exert more potent effects.

Effects of perfluoroalkyl acids on the function of the thyroid hormone and the aryl hydrocarbon receptor.
Long M., Ghisari M. and Bonefeld-Jorgensen E. C.
Environ Sci Pollut Res Int. 2013;20(11):8045-56.

Perfluoroalkyl acids (PFAAs) are perfluorinated compounds that widely exist in the environment and can elicit adverse effects including endocrine disruption in humans and animals. This study investigated the effect of seven PFAAs on the thyroid hormone (TH) system assessing the proliferation of the 3,3',5-triiodo-L-thryonine (T3)-dependent rat pituitary GH3 cells using the T-screen assay and the effect on the aryl hydrocarbon receptor (AhR) transactivation in the AhR-luciferase reporter gene bioassay. A dose-dependent impact on GH3 cells was observed in the range 1x10(-9)-1x10(-4) M: seven PFAAs (perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid, perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA)) inhibited the GH3 cell growth, and four PFAAs (PFOS, PFHxS, PFNA, and PFUnA) antagonized the T3-induced GH3 cell proliferation. At the highest test concentration, PFHxS showed a further increase of the T3-induced GH3 growth. Among the seven tested PFAAs, only PFDoA and PFDA elicited an activating effect on the AhR. In conclusion, PFAAs possess in vitro

endocrine-disrupting potential by interfering with TH and AhR functions, which need to be taken into consideration when assessing the impact on human health.

Mechanistic toxicity study of perfluorooctanoic acid in zebrafish suggests mitochondrial dysfunction to play a key role in PFOA toxicity.
Hagenaars A., Vergauwen L., Benoot D., Laukens K. and Knapen D.
Chemosphere. 2013;91(6):844-56.

The aquatic environment is an important site for perfluorooctanoic acid (PFOA) deposit. Nevertheless, the exact mode of action and its resulting toxicological effects in aquatic organisms remain largely unknown. To gain a better understanding of the mode of action of teleost PFOA toxicity, transcriptomics, proteomics, biochemical parameters and reproduction were integrated in this study. Male and female zebrafish were exposed to nominal concentrations of 0.1, 0.5 and 1 mg L(-1) PFOA for 4 and 28 d resulting in PFOA accumulation which was higher in males than in females. These gender-related differences were likely caused by different elimination rates due to distinct hormone levels and differences in transport activity by solute carriers. The general mode of action of PFOA was described as an increase of the mitochondrial membrane permeability followed by an impairment of aerobic ATP production. Depletion of liver glycogen stores together with altered expression levels of transcripts involved in carbohydrate metabolism, with emphasis on anaerobic metabolism, was probably a means of compensating for this decreased aerobic efficiency. The mitochondrial dysfunction further resulted in effects on oxidative stress and apoptosis at the gene transcript and protein level. As a consequence, evidence for the replacement of the affected cells and organelles to sustain tissue homeostasis was found at the transcript level, resulting in an even greater glycogen depletion. Despite this increase in metabolic expenditure, no effects on reproduction were found indicating that the fish seemed to cope with exposure to the tested concentrations of PFOA during the exposure period of 1 month.

Endocrine-related effects of perfluorooctanoic acid (PFOA) in zebrafish, H295R steroidogenesis and receptor reporter gene assays.
Du G., Huang H., Hu J., Qin Y., Wu D., Song L., Xia Y. and Wang X.
Chemosphere. 2013;91(8):1099-106.

Perfluorooctanoic acid (PFOA), a persistent perfluorinated compound, is distributed widely in wildlife and humans. Recent studies showed that PFOA is a suspected endocrine disruptor. But the results are somewhat contradictory and the mechanisms are unclear. In this study, we investigated the endocrine-related effects of PFOA using

a series of assays. The lower dose effect of PFOA on development and endocrine-related gene expression were assessed in a short-term zebrafish assay in vivo. To clarify the mechanism of PFOA, in vitro assays were performed. We tested the hormone receptor activities of ER, AR, and TR against PFOA using reporter gene assays. The hormone levels of estradiol (E2) and testosterone (T), the expression of major steroidogenic genes and the key steroidogenic gene regulator steroidogenic factors 1 (SF-1) were measured after PFOA exposure in H295R steroidogenesis assay. Exposure of zebrafish embryo to PFOA resulted in higher expression of esr1, hhex and pax. PFOA is able to interfere with hormone receptor ER and TR. In H295R cells, PFOA could increase the E2 production and decrease the T production, altered the expression of major steroidogenic genes and regulator SF-1. The current findings indicated the potential endocrine-related effects of PFOA and provided novel information for human risk assessment.

Changes in morphometry and association between whole-body fatty acids and steroid hormone profiles in relation to bioaccumulation patterns in salmon larvae exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids. Arukwe A., Cangialosi M. V., Letcher R. J., Rocha E. and Mortensen A. S. Aquatic toxicology (Amsterdam, Netherlands). 2013;130-131:219-30.

In the present study, we have used salmon embryos whose continuous exposure to waterborne PFOA or PFOS at 100 µg/L started as freshly fertilized eggs, and lasted for a total of 52 days. PFOS and PFOA were dissolved in methanol (carrier vehicle) whose concentration never exceeded 0.01% of total tank volume. Samples were collected at day 21, 28, 35, 52, 49 and 56 after the start of the exposure. Note that days 49 and 56 represent end of exposure and 1 week after a recovery period, respectively. Tissue bioaccumulations were determined by HPLC/MS/MS, steroid hormones, fatty acids (FAs) and lipids were determined by GC-MS, while mRNA expression levels of genes were determined by qPCR in whole body homogenate. We observed that PFOS and PFOA showed a steady increase in whole body burden during the exposure period, with a slight decrease after the recovery period. Calculated somatic indexes showed that PFOA produced increases in heart-, thymus-, liver- and kidney somatic indexes (HSI, TSI, LSI and KSI). PFOA and PFOS exposure produced significant decreases in whole body dehydroepiandrosterone (DHEA), estrone and testosterone at sampling day 21 and a strong increase of cortisol and cholesterol at the end of recovery period (day 56). PFOA and PFOS effects differed with DHEA and estrone. While PFOS decreased DHEA levels, PFOA produced an increase at day 49, and while PFOS decreased estrone, PFOA produced a slight increase at day 56. We observed changes in FA composition that predominantly involved increases in FA

methyl esters (FAMEs), mono- and poly-unsaturated FA (MUFA and PUFA) and a decrease in n-3/n-6 PUFA ratio by both PFOA and PFOS. Particularly, an increase in -pentadecenoic MUFA (15:1), two n-3 PUFAs α-linolenic acid [ALA: 18:3 n3] and eicosapentaenoic acid [EPA: 20:5 n-3] and n-6 PUFA: arachidonic acid [ARA: 20:4 n6], docosapentaenoic acid (DPA) by PFOA and PFOS were observed. These effects were associated with changes in mRNA expression of FA elongase (FAE), Δ5-desaturase (FAD5) and Δ6-desaturase (FAD6) genes. In summary, the changes in hormonal and FA profiles may represent cellular and/or physiological adaptation to continuous PFOS and PFOA exposure by increasing membrane fluidity, and/or overt developmental effects. The present findings provide some potential insights and basis for a better understanding on the possible mechanisms of PFCs toxicity in fish.

Perflurooctanoic acid induces developmental cardiotoxicity in chicken embryos and hatchlings.
Jiang Q., Lust R. M., Strynar M. J., Dagnino S. and DeWitt J. C.
Toxicology. 2012;293(1-3):97-106.

Perfluorooctanoic acid (PFOA) is a widespread environmental contaminant that is detectable in serum of the general U.S. population. PFOA is a known developmental toxicant that induces mortality in mammalian embryos and is thought to induce toxicity via interaction with the peroxisome proliferator activated receptor α (PPARα). As the cardiovascular system is crucial for embryonic survival, PFOA-induced effects on the heart may partially explain embryonic mortality. To assess impacts of PFOA exposure on the developing heart in an avian model, we used histopathology and immunohistochemical staining for myosin to assess morphological alterations in 19-day-old chicken embryo hearts after PFOA exposure. Additionally, echocardiography and cardiac myofibril ATPase activity assays were used to assess functional alterations in 1-day-old hatchling chickens following developmental PFOA exposure. Overall thinning and thinning of a dense layer of myosin in the right ventricular wall were observed in PFOA-exposed chicken embryo hearts. Alteration of multiple cardiac structural and functional parameters, including left ventricular wall thickness, left ventricular volume, heart rate, stroke volume, and ejection fraction were detected with echocardiography in the exposed hatchling chickens. Assessment of ATPase activity indicated that the ratio of cardiac myofibril calcium-independent ATPase activity to calcium-dependent ATPase activity was not affected, which suggests that developmental PFOA exposure may not affect cardiac energetics. In summary, structural and functional characteristics of the heart appear to be developmental

targets of PFOA, possibly at the level of cardiomyocytes. Additional studies will investigate mechanisms of PFOA-induced developmental cardiotoxicity.

Endocrine and developmental effects in Atlantic salmon (Salmo salar) exposed to perfluorooctane sulfonic or perfluorooctane carboxylic acids.
Spachmo B. and Arukwe A.
Aquat Toxicol. 2012;108:112-24.

In this study, we have investigated the effect of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on endocrine signalling, growth and development in Atlantic salmon (Salmo salar) embryos and larvae. Expression of genes related to the hypothalamic-pituitary-thyroid (HPT) axis, growth-hormone/insulin-like growth factor (GH/IGF) axis and the steroid hormone axis were used as indicators of endocrine disruption. We also studied bone development in larvae, both by observing skeletal structure formation and by investigating expression of genes involved in ossification process. Atlantic salmon embryos, kept in plastic tanks at 5-7 degrees C, were exposed to 100 mug/L PFOA or PFOS from egg stage for a period of 52 days, followed by one-week recovery period. Sampling was performed at day 21, 35, 49 and 56 representing age 549, 597, 679 and 721 dd (dd or day degrees = number of days x temperature in degree Celsius: degrees C). Note that day 56 or 721 dd is the end of the 1-week recovery period. Larvae were divided into designated head and body regions for the purpose of gene expression analysis, except for genes that regulate ossification that were analyzed in whole larvae. Expression of thyroid receptor α and β (TRα and TRβ), thyroid-stimulating hormone β (TSHβ), T(4) outer-ring deiodinase (T(4)ORD), growth hormone (GH), insulin-like growth factor-I and II (IGF-I and II), insulin-like growth factor I receptor (IGF-IR), and estrogen receptor α and β (ERα and ERβ) were investigated using quantitative PCR. Both PFOS and PFOA exposure produced non-significant alterations in larvae weight (except after the recovery period when a decrease was observed), while larvae length was unaffected. PFOS and PFOA exposure produced body- and head region-specific alterations in expression of all the investigated gene transcripts. Expression of IGF-I and IGF-IR paralleled that of GH, indicating that perturbation of GH expression is a possible end point for disruption of the GH-IGF axis. We did not observe developmental changes related to angiogenesis, ossification and chondrogenesis after exposure to PFOS and PFOA. Transcriptional abnormalities may serve as indicators of chronic exposure, although the concrete mechanisms causing the observed effects remain ambiguous. The implications of these findings for the complete lifecycle, including other developmental and/or reproductive damage, are areas of future study.

Effects of perfluorooctanoic acid (PFOA) on expression of peroxisome proliferator-activated receptors (PPAR) and nuclear receptor-regulated genes in fetal and postnatal CD-1 mouse tissues.
Abbott B. D., Wood C. R., Watkins A. M., Tatum-Gibbs K., Das K. P. and Lau C.
Reprod Toxicol. 2012;33(4):491-505.

PPARs regulate metabolism and can be activated by environmental contaminants such as perfluorooctanoic acid (PFOA). PFOA induces neonatal mortality, developmental delay, and growth deficits in mice. Studies in genetically altered mice showed that PPARα is required for PFOA-induced developmental toxicity. In this study, pregnant CD-1 mice were dosed orally from GD1 to 17 with water or 5mg PFOA/kg to examine PPARα, PPARβ, and PPARγ expression and profile the effects of PFOA on PPAR-regulated genes. Prenatal and postnatal liver, heart, adrenal, kidney, intestine, stomach, lung, spleen, and thymus were collected at various developmental ages. RNA and protein were examined using qPCR and Western blot analysis. PPAR expression varied with age in all tissues, and in liver PPARα and PPARgamma expression correlated with nutritional changes as the pups matured. As early as GD14, PFOA affected expression of genes involved in lipid and glucose homeostatic control. The metabolic disruption produced by PFOA may contribute to poor postnatal survival and persistent weight deficits of CD-1 mouse neonates.

Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model.
Loccisano A. E., Campbell J. L., Jr., Butenhoff J. L., Andersen M. E. and Clewell H. J., 3rd
Reprod Toxicol. 2012;33(4):468-90.

Perfluoroalkyl carboxylates and sulfonates (PFAAs) have many consumer and industrial applications. Developmental toxicity studies in animals have raised concern about potential developmental effects of PFOA and PFOS in humans. We have developed PBPK models for PFAAs in the rat to help define a relationship between external dose, internal tissue concentrations, and observed adverse effects, and to understand how physiological changes that occur during gestation and lactation affect tissue distribution of PFAAs in the mother, fetus, and neonate. The models developed here expand upon a PBPK model for PFAAs in the adult female rat, and are consistent with available PK data. These models, along with the adult rat PFAA models, published in the companion paper, will help address concerns about possible health

effects due to PFAA exposure in the fetus and neonate and will be useful in comparing PK across life stages.

Effects of per- and polyfluorinated compounds on adult rat testicular cells following in vitro exposure.
Lindeman B., Maass C., Duale N., Gutzkow K. B., Brunborg G. and Andreassen A.
Reprod Toxicol. 2012;33(4):531-7.

Testicular toxicity is observed following exposure of rats to per- and polyfluorinated compounds (PFCs). Such compounds were also shown to induce oxidative stress and changes in ABC efflux transporters e.g. P-gp, implying two mechanisms which may contribute to testicular toxicity. We studied the toxicity of four PFCs (PFOA, PFNA, 8:2 FTOH and 6:2 FTOH) on primary rat testicular cells. DNA damage was studied by the comet assay including Fpg enzyme treatment to detect oxidative lesions. The levels of the ABC efflux transporters Bcrp1, Oat2 and P-gp were studied by real-time RT-PCR or flow cytometry. A PFNA associated increase in DNA SSBs was attributed to a subpopulation of moderately damaged cells possibly associated with cytotoxicity. No significant increase in oxidative DNA damage was measured for any of the PFCs. Expression levels of ABC efflux transporters suggest that PFCs may increase expression levels of the P-gp protein and the Oat2 gene.

Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model.
Loccisano A. E., Campbell J. L., Jr., Butenhoff J. L., Andersen M. E. and Clewell H. J., 3rd
Reprod Toxicol. 2012;33(4):452-67.

Perfluoroalkyl acid carboxylates and sulfonates (PFAAs) have many consumer and industrial applications. The persistence and widespread distribution of PFAAs have brought them under intense scrutiny. Limited PK data for PFAAs is available for humans; however, toxicological and pharmacokinetic data exist for rats, which can be useful for cross-species extrapolation. In this work, PBPK models were developed for adult male and female rats to describe the pharmacokinetics of PFOA and PFOS. The models contain a description of saturable renal resorption, free fraction of chemical in plasma, and saturable binding in liver. Both male and female rat models for each chemical were consistent with available PK data resulting from IV, oral, and dietary dosing regimens. Predicted plasma concentration curves followed trends observed in experimental data, and model predictions were within a factor of two of experimental values. PFOA and PFOS rat model output is sensitive to parameters governing renal

resorption, indicating that renal resorption is responsible for the long-half life. These models, along with the PFAA gestation and lactation models published in this issue, will help address concerns about possible health effects due to PFAA exposure in the fetus and neonate and will be useful in comparing PK across life stages.

Transcription of genes involved in fat metabolism in chicken embryos exposed to the peroxisome proliferator-activated receptor α (PPAR-α;) agonist GW7647 or to perfluorooctane sulfonate (PFOS) or perfluorooctanoic acid (PFOA).
Stromqvist M., Olsson J. A., Karrman A. and Brunstrom B.
Comparative Biochemistry and Physiology - Part C: Toxicology & Pharmacology. 2012;156(1):29-36.

Perfluoroalkyl acids (PFAAs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are developmental toxicants in various animal classes, including birds. Both compounds interact with peroxisome proliferator-activated receptors (PPARs), but it is not known whether activation of PPARs is involved in their embryo toxicity in birds. We exposed chicken embryos via egg injection at a late developmental stage to GW7647, a potent PPARα agonist in mammals, and to PFOS or PFOA. Mortality was induced by PFOS and PFOA but not by GW7647. Transcripts of a number of genes activated by PPARα agonists in mammals were analyzed in liver and kidney of 18-day-old embryos. Several of the genes were induced in both liver and kidney following exposure to GW7647. Treatment with PFOA resulted in induction of acyl-coenzyme A oxidase mRNA in liver, whereas none of the genes were significantly induced by PFOS treatment. No up-regulation of gene transcription was found in kidney following treatment with PFOS or PFOA. Principal component analysis showed that PFOA caused an mRNA expression pattern in liver more similar to the pattern induced by GW7647 than PFOS did. Our findings do not support that the embryo mortality by PFOS and PFOA in chicken embryos involves PPARα activation.

Effects of per- and polyfluorinated compounds on adult rat testicular cells following in vitro exposure.
Lindeman B., Maass C., Duale N., Gutzkow K. B., Brunborg G. and Andreassen A.
Reproductive toxicology (Elmsford, NY). 2012;33(4):531-7.

Testicular toxicity is observed following exposure of rats to per- and polyfluorinated compounds (PFCs). Such compounds were also shown to induce oxidative stress and changes in ABC efflux transporters e.g. P-gp, implying two mechanisms which may contribute to testicular toxicity. We studied the toxicity of four PFCs (PFOA, PFNA, 8:2 FTOH and 6:2 FTOH) on primary rat testicular cells. DNA damage was studied by the

comet assay including Fpg enzyme treatment to detect oxidative lesions. The levels of the ABC efflux transporters Bcrp1, Oat2 and P-gp were studied by real-time RT-PCR or flow cytometry. A PFNA associated increase in DNA SSBs was attributed to a subpopulation of moderately damaged cells possibly associated with cytotoxicity. No significant increase in oxidative DNA damage was measured for any of the PFCs. Expression levels of ABC efflux transporters suggest that PFCs may increase expression levels of the P-gp protein and the Oat2 gene.

Ammonium perfluorooctanoate may cause testosterone reduction by adversely affecting testis in relation to PPARα.
Li Y., Ramdhan D. H., Naito H., Yamagishi N., Ito Y., Hayashi Y., Yanagiba Y., Okamura A., Tamada H., Gonzalez F. J. and Nakajima T.
Toxicol Lett. 2011;205(3):265-72.

Perfluorooctanoate, a peroxisome proliferator-activated receptor α (PPARα) agonist, has the potential to lower testosterone levels as a result of testicular toxicity. To elucidate the mechanism and impact of PPARα on this reproductive toxicity, ammonium perfluorooctanoate (APFO) at doses of 0, 1.0 (low) mg/kg/day, or 5.0 (high) mg/kg/day was orally given daily to 129/sv wild-type (mPPARα), Pparα-null and PPARα-humanized (hPPARα) mice for 6 weeks. Both low- and high-dose APFO significantly reduced plasma testosterone concentrations in mPPARα and hPPARα mice, respectively. These decreases may, in part, be associated with decreased expression of mitochondrial cytochrome P450 side-chain cleavage enzyme, steroidogenic acute regulatory protein or peripheral benzodiazepine receptor as well as microsomal cytochrome P450(17α) involved in the steroidogenesis. Additionally, both doses increased abnormalities in sperm morphology and vacuolated cells in the seminiferous tubules of both mouse lines. In contrast, APFO caused only a marginal effect either on the testosterone synthesis system or sperm and testis morphology in Pparα-null mice. These results suggest that APFO may disrupt testosterone biosynthesis by lowering the delivery of cholesterol into the mitochondria and decreasing the conversion of cholesterol to pregnenolone and androstandione in the testis of mPPARα and hPPARα mice, which may, in part, be related to APFO-induced mitochondrial damage.

Tissue bioaccumulation patterns, xenobiotic biotransformation and steroid hormone levels in Atlantic salmon (Salmo salar) fed a diet containing perfluoroactane sulfonic or perfluorooctane carboxylic acids.
Mortensen A. S., Letcher R. J., Cangialosi M. V., Chu S. and Arukwe A.
Chemosphere. 2011;83(8):1035-44.

In the present study, groups of juvenile Atlantic salmon (Salmo salar) were fed gelatine capsules containing fish-food spiked with PFOA or PFOS (0.2 mg kg(-1) fish) and solvent (methanol). The capsules were given at days 0, 3 and 6. Blood, liver and whole kidney samples were collected prior to exposure (no solvent control), and at days 2, 5, 8 and 14 after exposure (Note: that day 14 after exposure is equal to 7d recovery period). We report on the differences in the tissue bioaccumulation patterns of PFOS and PFOA, in addition to tissue and compound differences in modulation pattern of biotransformation enzyme genes. We observed that the level of PFOS and PFOA increased in the blood, liver and kidney during the exposure period. Different PFOS and PFOA bioaccumulation patterns were observed in the kidney and liver during exposure- and after the recovery periods. Particularly, after the recovery period, PFOA levels in the kidney and liver tissues were almost at the control level. On the contrary, PFOS maintained an increase with tissue-specific differences, showing a higher bioaccumulation potential (also in the blood), compared with PFOA. While PFOS and PFOA produced an apparent time-dependent increase in kidney CYP3A, CYP1A1 and GST expression, similar effects were only temporary in the liver, significantly increasing at sampling day 2. PFOA and PFOS exposure resulted in significant decreases in plasma estrone, testosterone and cortisol levels at sampling day 2, and their effects differed with 17α-methyltestostrerone showing significant decrease by PFOA (also for cholesterol) and increase by PFOS. PFOA significantly increased estrone and testosterone, and no effects were observed for cortisol, 17α-methyltestosterone and cholesterol at sampling day 5. Overall, the changes in plasma steroid hormone levels parallel changes in CYP3A mRNA levels. Given that there are no known studies that have demonstrated such tissue differences in bioaccumulation patterns with associated differences in toxicological responses in any fish species or lower vertebrate, the present findings provide some potential insights and basis for a better understanding of the possible mechanisms of PFCs toxicity that need to be studied in more detail.

Disturbance of perfluorooctanoic acid on development and behavior in Drosophila larvae.
Wang J., Li Y., Liu Y., Zhang H. and Dai J.
Environ Toxicol Chem. 2010;29(9):2117-22.

Perfluorooctanoic acid (PFOA) is a well-known perfluorinated compound (PFC), and its toxicological impact is currently of worldwide concern. In this study, we sought to evaluate the potential biological effects and modes of action of PFOA in a range of physiologically and developmentally related phenotypes in the fruit fly Drosophila melanogaster. The results clearly indicated that the toxic effects of PFOA at the organismal level were associated with the developmental status of the organism, with larvae being most sensitive to this chemical. Except for the decreased weight of both sexes and the reduced longevity of male adults, PFOA had a robust effect on larval development, as determined by reduced body volume, aberrant foraging behavior, molting arrest, and polyphasic lethality. Remarkably, nutrient supplementation of the diet efficiently rescued the lethal effect of high PFOA concentrations on larval development. This result indicated that PFOA probably competed with nutritional components, leading to a disruption of the metabolic pathways responsible for larval development.

Inhibition of 3β- and 17β-hydroxysteroid dehydrogenase activities in rat Leydig cells by perfluorooctane acid.
Zhao B., Chu Y., Hardy D. O., Li X. K. and Ge R. S.
J Steroid Biochem Mol Biol. 2010;118(1-2):13-7.

Perfluorooctane acid (PFOA) is classified as a persistent organic pollutant and as an endocrine disruptor. The mechanism by which PFOA causes reduced testosterone production in males is not known. We tested our hypothesis that PFOA interferes with Leydig cell steroidogenic enzymes by measuring its effect on 3β-hydroxysteroid dehydrogenase (3β-HSD) and 17β-hydroxysteroid dehydrogenase 3 (17β-HSD3) activities in rat testis microsomes and Leydig cells. The IC(50)s of PFOA and mode of inhibition were assayed. PFOA inhibited microsomal 3β-HSD with an IC(50) of 53.2+/-25.9 microM and 17β-HSD3 with an IC(50) 17.7+/-6.8 microM. PFOA inhibited intact Leydig cell 3β-HSD with an IC(50) of 146.1+/-0.9 microM and 17β-HSD3 with an IC(50) of 194.8+/-1.0 microM. The inhibitions of 3β-HSD and 17β-HSD3 by PFOA were competitive for the substrates. In conclusion, PFOA inhibits 3β-HSD and 17β-HSD3 in rat Leydig cells.

Pipping success and liver mRNA expression in chicken embryos exposed in ovo to C8 and C11 perfluorinated carboxylic acids and C10 perfluorinated sulfonate.
O'Brien J. M., Crump D., Mundy L. J., Chu S., McLaren K. K., Vongphachan V., Letcher R. J. and Kennedy S. W.
Toxicol Lett. 2009;190(2):134-9.

Several perfluoroalkyl compounds (PFCs) are ubiquitous environmental contaminants that can biomagnify in species at high trophic levels including wild birds. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have been detected in wild birds and are known to reduce hatching success of laboratory-exposed chicken embryos at environmentally relevant concentrations. Limited toxicity data are available regarding avian exposure to PFCs of chain lengths greater than C(8), which are of increasing environmental relevance following the recent phase-out of PFOS and PFOA. In this study, linear PFOA, perfluoroundecanoic acid (PFUdA) and perfluorodecane sulfonate (PFDS) were injected into the air cell of white leghorn chicken eggs (Gallus gallus domesticus) prior to incubation to determine effects on embryo pipping success. Furthermore, mRNA expression of key genes involved in pathways implicated in PFC toxicity was monitored in liver tissue. PFOA, PFUdA or PFDS had no effect on embryonic pipping success at concentrations up to 10 microg/g. All PFCs accumulated in the liver to concentrations greater than the initial whole-egg concentration as determined by HPLC/MS/MS. Hepatic accumulation was highest for PFOA (4.5 times) compared to PFUdA and PFDS. Cytochrome P450 1A4 and liver fatty acid binding protein mRNA expression increased after exposure to PFUdA but was only statistically significant at 10 microg/g; several orders of magnitude higher than levels found in wild bird eggs. Based on the present results for white leghorn chickens, current environmental concentrations of PFOA, PFUdA and PFDS are unlikely to affect the hatching success of wild birds.

Pharmacokinetic modeling of perfluorooctanoic acid during gestation and lactation in the mouse.
Rodriguez C. E., Setzer R. W. and Barton H. A.
Reprod Toxicol. 2009;27(3-4):373-86.

Perfluorooctanoic acid (PFOA) is a processing aid for the polymerization of commercially valuable fluoropolymers. Its widespread environmental distribution, presence in human blood, and adverse effects in animal toxicity studies have triggered attention to its potential adverse effects to humans. PFOA is not metabolized and exhibits dramatically different serum/plasma half-lives across species. Estimated half-lives for humans, monkeys, mice, and female rats are 3-5 years, 20-30 days, 12-20

days, and 2-4h, respectively. Developmental toxicity is one of the most sensitive adverse effects associated with PFOA exposure in rodents, but its interpretation for risk assessment is currently hampered by the lack of understanding of the inter-species pharmacokinetics of PFOA. To address this uncertainty, a biologically supported dynamic model was developed whereby a two-compartment system linked via placental blood flow described gestation and milk production linked a lactating dam to a growing pup litter compartment. Postnatal serum levels of PFOA for 129S1/SvImJ mice at doses of 1mg/kg or less were reasonably simulated while prenatal and postnatal measurements for CD-1 mice at doses of 1mg/kg or greater were simulated via the addition of a biologically based saturable renal resorption description. Our results suggest that at low doses a linear model may suffice for describing the pharmacokinetics of PFOA while a more complex model may be needed at higher doses. Although mice may appear more sensitive based on administered dose of PFOA, the internal dose metrics estimated in this analysis indicate that they may be equal or less sensitive than rats.

Analysis of PFOA in dosed CD-1 mice. Part 2. Disposition of PFOA in tissues and fluids from pregnant and lactating mice and their pups.
Fenton S. E., Reiner J. L., Nakayama S. F., Delinsky A. D., Stanko J. P., Hines E. P., White S. S., Lindstrom A. B., Strynar M. J. and Petropoulou S. S.
Reprod Toxicol. 2009;27(3-4):365-72.

Previous studies in mice with multiple gestational exposures to perfluorooctanoic acid (PFOA) demonstrate numerous dose dependent growth and developmental effects which appeared to worsen if offspring exposed in utero nursed from PFOA-exposed dams. To evaluate the disposition of PFOA in the pregnant and lactating dam and her offspring, time-pregnant CD-1 mice received a single 0, 0.1, 1, or 5mg PFOA/kg BW dose (n=25/dose group) by gavage on gestation day 17. Maternal and pup fluids and tissues were collected over time. Pups exhibited significantly higher serum PFOA concentrations than their respective dams, and their body burden increased after birth until at least postnatal day 8, regardless of dose. The distribution of milk:serum PFOA varied by dose and time, but was typically in excess of 0.20. These data suggest that milk is a substantial PFOA exposure route in mice and should be considered in risk assessment modeling designs for this compound.

Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid.
Rosen M. B., Schmid J. E., Das K. P., Wood C. R., Zehr R. D. and Lau C.
Reprod Toxicol. 2009;27(3-4):278-88.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are environmental contaminants found in the tissues of humans and wildlife. They are activators of peroxisome proliferator-activated receptor-α (PPAR α) and exhibit hepatocarcinogenic potential in rats. PFOS and PFOA are also developmental toxicants in rodents and PFOS has been shown to induce pulmonary deficits in rat offspring. Pregnant CD-1 mice were dosed with 0, 5, or 10mg/kg PFOS from gestation days 1-17. Transcript profiling was conducted on the fetal liver and lung. Results were contrasted to data derived from a previous PFOA study. PFOS-dependent changes were primarily related to activation of PPAR α. No remarkable differences were found between PFOS and PFOA. Given that PPAR α signaling is required for neonatal mortality in PFOA-treated mice but not those exposed to PFOS, the neonatal mortality observed for PFOS may reflect functional deficits related to the physical properties of the chemical rather than to transcript alterations.

Analysis of PFOA in dosed CD1 mice. Part 1. Methods development for the analysis of tissues and fluids from pregnant and lactating mice and their pups.
Reiner J. L., Nakayama S. F., Delinsky A. D., Stanko J. P., Fenton S. E., Lindstrom A. B. and Strynar M. J.
Reprod Toxicol. 2009;27(3-4):360-4.

The number of studies involving the analysis of perfluorooctanoic acid (PFOA) has increased recently because PFOA is routinely detected in human blood samples from around the world. Recent studies with mice have shown that dosing pregnant dams with PFOA during gestation gives rise to a dose-dependent mortality in the litters, a reduction in neonatal body weight for the surviving pups, and subsequent deficits in mammary gland development when compared to control animals. The actual body burdens of PFOA in dams and pups associated with these endpoints have not been determined, in part due to a lack of robust analytical methods for these matrices. The goal of the current study was to develop reliable methods with acceptable performance characteristics for the analysis of PFOA in several matrices relevant to pregnant mouse studies. Dam and pup serum, amniotic fluid, urine, milk, mammary tissue, and whole mouse pups were isolated for method development and analysis. The resulting method provided excellent accuracy (92.1-111%) and reproducibility (relative standard

deviation 4.3-21%) making them very useful for future studies. These methods were then applied to dosed animal fluids and tissues in order to conduct a thorough evaluation of the pharmacokinetics in utero. Resulting tissue specific measurements of PFOA in serum, amniotic fluid, urine, milk, mammary tissue, and whole pup homogenate will be used to more completely describe the dose-response relationships for the most sensitive health outcomes and inform pharmacokinetic models that are being developed and evaluated.

Exposure of developing chicks to perfluorooctanoic acid induces defects in prehatch and early posthatch development.
Yanai J., Dotan S., Goz R., Pinkas A., Seidler F. J., Slotkin T. A. and Zimmerman F.
J Toxicol Environ Health A. 2008;71(2):131-3.

There is increasing concern over the widespread use of perfluorinated chemicals, which accumulate in various tissues and penetrate the mammalian fetus. A chick model was established for the rapid evaluation of teratogenicity of these chemicals, an important issue because developmental defects often occur at lower exposures than those required for adult systemic toxicity. Chicken eggs were injected with varying doses of perfluorooctanoic acid prior to incubation. Observed were defects in hatching, increased incidence of splayed legs, and interference with the appropriate development of yellow plumage. All these defects are potentially related to essential molecular/biochemical and functional development of the chick. Because of the relationship between structural defects and vulnerability of the developing brain, our model points to the need to evaluate neurobehavioral teratogenicity, which may involve even lower doses.

[Researching progresses in environmental pollution behavior, toxic effects and mechanisms of PFOS/PFOA].
Zhou Q. X. and Hu X. G.
Huan Jing Ke Xue. 2007;28(10):2153-62.

It has been validated that perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) can be considered as emerging persistent organic pollutants. In recent years, there are increasing distribution of PFOS/PFOA in environmental systems, and accumulation and toxic effects of PFOS/PFOA in living organisms. Thus, environmental pollution and biological exposure of PFOS/PFOA were firstly analyzed on the basis of their pollution levels, exposure to wild animals, exposure to human bodies, and changing trends in pollution and exposure. Secondly, movement and transformation behaviors of PFOS/PFOA in environment were expounded on the basis

of their transport and transformation processes in air environment, wastewater and sewage sludge, and their accumulation, metabology and degradation processes in living organisms. Some recent important advances in ecological effects of PFOS/PFOA and their possible mechanisms were summarized. Finally, the future emphases of research on pollution ecology of PFOS/PFOA were tentatively suggested.

Estrogen-like properties of perfluorooctanoic acid as revealed by expressing hepatic estrogen-responsive genes in rare minnows (Gobiocypris rarus).
Wei Y., Dai J., Liu M., Wang J., Xu M., Zha J. and Wang Z.
Environ Toxicol Chem. 2007;26(11):2440-7.

Perfluorooctanoic acid (PFOA) is an important perfluorinated compound (PFC) with various applications and has been widely disseminated in the environment, wildlife, and humans. The present study investigated the effects of waterborne PFOA on the expression of hepatic estrogen-responsive genes, vitellogenin (VTG), and estrogen receptor β (ERβ) and on the gonadal development in a freshwater rare minnow (Gobiocypris rarus). The mRNA levels of VTG and ERβ were determined using reverse transcription polymerase chain reaction (RT-PCR) techniques, and VTG protein levels were identified using enzyme-linked immunosorbent assay. A significant increase of VTG expression in the livers of both mature males and females was observed after 14 and 28 d of exposure to 3, 10, and 30 mg/L PFOA, indicating that PFOA could induce VTG synthesis. The expression of ERβ increased significantly in livers of both mature males and females after a 14-d exposure, although no difference was observed after a 28-d exposure. The development of oocytes in testes exposed to PFOA also provided evidence of estrogenic activity in males. The ovaries of PFOA-exposed females underwent degeneration, as reported in other fish species exposed to environmental estrogens. This preliminary study indicates that PFOA can disturb the activity of estrogen in mature male rare minnows by inducing hepatic estrogen-responsive genes, VTG and ERβ, and barrier female reproduction.

Perfluorooctanoic acid and perfluorononanoic acid in fetal and neonatal mice following in utero exposure to 8-2 fluorotelomer alcohol.
Henderson W. M. and Smith M. A.
Toxicol Sci. 2007;95(2):452-61.

8-2 Fluorotelomer alcohol (FTOH) and its metabolites, perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), are developmental toxicants but metabolism and distribution during pregnancy are not known. To examine this, timed-pregnant mice

received a single gavage dose (30 mg 8-2 FTOH/kg body weight) on gestational day (GD) 8. Maternal and neonatal serum and liver as well as fetal and neonatal homogenate extracts were analyzed using gas chromatography coupled with mass spectrometry. During gestation (GD9 to GD18), maternal serum and liver concentrations of PFOA decreased from 789 +/- 41 to 668 +/- 23 ng/ml and from 673 +/- 23 to 587 +/- 55 ng/g, respectively. PFOA was transferred to the developing fetuses as early as 24-h posttreatment with concentrations increasing from 45 +/- 9 ng/g (GD10) to 140 +/- 32 ng/g (GD18), while PFNA was quantifiable only at GD18 (31 +/- 4 ng/g). Post-partum, maternal serum PFOA concentrations decreased from 451 +/- 21 ng/ml postnatal day (PND) 1 to 52 +/- 19 ng/ml (PND15) and PFNA concentrations, although fivefold less, exhibited a similar trend. Immediately after birth, pups were cross-fostered with dams that had been treated during gestation with 8-2 FTOH (T) or vehicle (C) resulting in four treatment groups in which the first letter represents in utero (fetal) exposure and the second represents lactational (neonatal) exposure: C/C, T/C, C/T, T/T. On PND1, neonatal whole-body homogenate concentrations of PFOA from T/T and T/C groups averaged 200 +/- 26 ng/g, decreased to 149 +/- 19 ng/g at PND3 and this decreasing trend was seen in both neonatal liver and serum from PND3 to PND15. Based on detectible amounts of PFOA in neonatal serum in the C/T group on PND3 (57 +/- 11 ng/ml) and on PND15 (58 +/- 3 ng/ml), we suggest that the neonates were exposed through lactation. In conclusion, exposure of neonates to PFOA and PFNA occurs both pre- and postnatally following maternal 8-2 FTOH exposure on GD8.

Perfluorooctanoic Acid (PFOA) and Perfluorononanoic Acid (PFNA) in Neonatal Mice Following In Utero Exposure to 8-2 Fluorotelomer Alcohol (FTOB).
Henderson W. M. and Smith M. A.
Birth Defects Res A Clin Mol Teratol. 2006;76(5):384.

The fluorotelomer alcohols (FTOHs) are the probable precursors of a homologous series of perfluoroalkyl carboxcyclic acids (PFCAs) detected globally in both mammalian and environmental samples. Recently, 8-2 FTOH has been classified as a xenoestrogen and its derivatives, perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), have been correlated with developmental toxicity in rats and rabbits. Previous work in our laboratory determined that in mice fetal concentrations of the terminal metabolites, PFOA and PFNA, increase up to gestation day (GD) 18 following a single oral maternal exposure on GD8. However, maternal serum and liver concentrations gradually decreased having reached peak concentrations by 24 h post-treatment. The purpose of this study was to investigate the neonatal transfer of these stable metabolites following in utero exposure to 8-2 FTOH.

Pregnant CD-1 mice were gavaged with 30 mg 8-2 FTOH/kg BW on GD8. Following parturition (postnatal day (PND) 0), half of the litters were cross-fostered with dams treated with vehicle control resulting in four treatment groups: no exposure (C/C), in utero exposure only (T/C), postnatal exposure (via lactation) (C/T), and both in utero and postnatal exposure (T/T). Animals were sacrificed PND 1, 3, and 15 and samples were collected for PFCA analysis. Maternal serum concentrations of PFOA decreased from 450+/-21 ng/mL on PND1 to 52+/-19 ng/mL on PND15. PFNA concentrations, although 10-fold less, exhibited a similar trend reaching concentrations below method detection limits at or before PND15. On PND1, neonatal tissue concentrations of PFOA from T/T and T/C groups were 144+/-6 and 128+/-9 ng/mg, respectively, and had significantly decreased ($p < 0.05$) by PND3 to 119+/-16 and 48+/-12 ng/mg, respectively. Similarly, group T/T neonatal serum concentrations of PFOA on PND3 were 469+/-21 ng/mL and on PND15 were 275+/-13 ng/mL, while group TIC concentrations were 417+/-16 and 140+/-22 ng/mL on PND3 and PND15, respectively. Neonates appear to be exposed through lactation based on detectible amounts of PFOA in neonatal serum on PND3 (52+/-8 ng/mL) in the C/T group. In conclusion, exposure of neonates to metabolites of 8-2 FTOH can occur both pre- and postnatally following a single oral maternal dose on GD8.

Perfluorooctanoate: Placental and lactational transport pharmacokinetics in rats. Hinderliter P. M., Mylchreest E., Gannon S. A., Butenhoff J. L. and Kennedy G. L., Jr. Toxicology. 2005;211(1-2):139-48.

This study was conducted to develop a quantitative understanding of the potential for gestational and lactational transfer of perfluorooctanoate (PFOA) in the rat. Time-mated female rats were dosed by oral gavage once daily at concentrations of 3, 10, or 30 mg/kg/day of the ammonium salt of PFOA (APFO) starting on gestation (G) day 4 and continuing until sacrifice. On days 10, 15, and 21G, five rats per dose level were sacrificed and blood samples were collected 2h post-dose. Embryos were collected on day 10G, amniotic fluid, placentas, and embryos/fetuses were collected on days 15 and 21G, and fetal blood samples were collected on day 21G. Five rats per dose level were allowed to deliver and nurse their litters, and on days 3, 7, 14, and 21 post-partum (PP) milk and blood samples of maternal and pup were collected 2h post-dose. All samples were analyzed by high-performance liquid chromatography-mass spectrometry (HPLC-MS) for PFOA concentration. Concentrations of PFOA in maternal plasma and milk attained steady state during the sampling interval. The steady-state concentrations in maternal plasma were 10-15, 25-30, and 60-75 microg/mL in rats receiving 3, 10, and 30 mg/kg, respectively. Steady-state concentrations in milk were approximately 10 times less than those in maternal

plasma. The concentration of PFOA in fetal plasma on day 21G was approximately half the steady-state concentration in maternal plasma. The milk concentrations appeared to be generally comparable to the concentrations in pup plasma. Pup plasma concentrations decreased from day 3PP to day 7PP, and were similar on days 7, 14, and 21PP at all dose levels. PFOA was detected in placenta (days 15 and 21G), amniotic fluid (days 15 and 21G), embryo (days 10 and 15G), and fetus (day 21G). These pharmacokinetics allow estimation of the dose to developing and nursing rat offspring following maternal exposure.

Toxicity of perfluorooctane sulfonic acid and perfluorooctanoic acid to Chironomus tentans.
MacDonald M. M., Warne A. L., Stock N. L., Mabury S. A., Solomon K. R. and Sibley P. K.
Environ Toxicol Chem. 2004;23(9):2116-23.

Two perfluorinated surfactants, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), were evaluated for their toxicity to the aquatic midge, Chironomus tentans. Impetus for this laboratory study originated from a 10-d, in situ field assessment in which C. tentans was exposed to PFOS at concentrations ranging from 300 to 30,000 microg/L. No midges survived these exposures. Midge survival in a preliminary, acute 10-d laboratory test with nominal PFOS concentrations ranging from 0.1 to 100,000 microg/L showed similar toxicity with respect to survival (median lethal concentration [LC50], 45.2 microg/L) and growth (median effective concentration [EC50], 27.4 microg/L). A parallel test using PFOA indicated no significant impacts on survival or growth. A definitive 10-d assay with PFOS concentrations ranging from 1 to 150 microg/L produced an EC50 for growth (87.2+/-11.6 microg/L) of the same order of magnitude as that in the preliminary findings. The same was not true for survival, however, with the LC50 falling outside the range of test concentrations. To further investigate the sensitivity of C. tentans to PFOS, we conducted a chronic life-cycle test using a nominal concentration range of 1 to 100 microg/L. Three of the four endpoints measured-survival, growth, and emergence-were significantly affected, with EC50 values of 92.2+/-3.1, 93.8+/-2.6, and 94.5+/-3.2 microg/L, respectively. Reproduction was not affected by those PFOS concentrations at which females emerged. The results of the present study indicate that PFOS toxicity thresholds for C. tentans are as much as three orders of magnitude lower than those reported for other aquatic organisms but, at present, are approximately two orders of magnitude higher than those concentrations typically observed in aquatic environments.

Impact of perfluorooctanoic acid on fathead minnow (Pimephales promelas) fatty acyl-CoA oxidase activity, circulating steroids, and reproduction in outdoor microcosms.
Oakes K. D., Sibley P. K., Solomon K. R., Mabury S. A. and Van der Kraak G. J.
Environ Toxicol Chem. 2004;23(8):1912-9.

This study investigates reproductive impairment and biochemical changes in fathead minnow (Pimephales promelas) exposed for 39 d to varying concentrations of perfluorooctanoic acid (PFOA) under microcosm conditions. While the concentrations tested in this study were much higher than those normally found in the environment, no mortality was associated with PFOA exposure. Only modest changes were observed in condition factor and in relative liver and gonad size. Significant declines in circulating plasma steroids were observed, but these were accompanied by only limited increases in time to first oviposition and decreases in overall egg production. Peroxisome proliferation, as quantified by fatty acyl-CoA oxidase (FAO) activity, was elevated with low PFOA concentrations but attenuated with exposure to higher PFOA doses. Little evidence was seen of differential induction of peroxisome-associated enzyme activity with sex. Oxidative stress, as quantified by the 2-thiobarbituric acid reactive substances (TBARS) assay, was only modestly influenced by PFOA exposure and is not a significant consequence of FAO activity in fathead minnow. Perfluorooctanoic acid appears to be relatively nontoxic at environmentally relevant concentrations but may impact biochemical and reproductive endpoints under conditions associated with environmental spills.

D. Titles only (abstracts not available)

Retraction. Prenatal PFOA exposure alters gene expression pathways in murine mammary gland.
Toxicological sciences : an official journal of the Society of Toxicology.
2015;145(1):211.

APPENDIX: PERFLUOROOCTANE SULFONATE

Perfluorooctane sulfonate (PFOS: CAS# 1763-23-1) is a synthetic, fully fluorinated organic compound with a long carbon chain. PFOS has lipid- and water-repellent properties and was used to produce a wide range of products, including fabric stain repellants, coatings for leather and paper products, fire-fighting foams, and mist-suppressants in acid baths. Although PFOS chemicals are no longer manufactured in the US, a few limited uses are allowed, and PFOS is still produced in China. PFOS resists typical environmental degradation processes and therefore persists in the environment. PFOS can also be formed by environmental degradation or metabolism from a large group of precursors.

This document presents a compilation of abstracts of articles on the developmental and reproductive toxicity of PFOS identified during our epidemiological screen and subsequent toxicological evaluation. OEHHA originally screened PFOS in 2007 but there was not sufficient human data available for PFOS to pass the screen at that time. We applied an epidemiologic data screen on PFOS in 2015. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. PFOS passed the epidemiologic screen. We also conducted a preliminary toxicological evaluation searching for relevant studies, including animal studies.

OEHHA used the information in this document to select nickel for presentation to the Developmental and Reproductive Toxicant Identification Committee as a possible candidate for Committee consideration. The abstracts compiled below are from epidemiologic and animal toxicity studies reporting on developmental and reproductive sequelae related to exposure to PFOS, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species).

Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Thirty-one epidemiologic studies of PFOS reporting increased risk of adverse developmental or reproductive outcomes were identified, fifteen of which were analytical studies of adequate quality. Four additional epidemiologic studies reported increased risk of adverse developmental or reproductive outcomes; however, the findings were not statistically significant. Twenty-nine epidemiologic studies reported no increased risk of adverse developmental or

reproductive outcomes as well as three studies which had unclear findings. Fifty-six related articles were also identified.

In addition, the following animal toxicity studies were identified.

- Twenty-nine animal studies of PFOS reporting reproductive or developmental toxicity were identified. One study reporting no reproductive or developmental toxicity was identified. Thirty-two related articles were also identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

i. Studies which were statistically significant

[*]Prenatal Exposure to Perfluoroalkyl Acids and Serum Testosterone Concentrations at 15 Years of Age in Female ALSPAC Study Participants.
Maisonet M., Calafat A. M., Marcus M., Jaakkola J. J. and Lashen H.
Environ Health Perspect. 2015 Jun 2. [Epub ahead of print].

BACKGROUND: Exposure to perfluorooctane sulfonic acid (PFOS) or to perfluorooctanoic acid (PFOA) increases mouse and human PPARalpha subtype activity which influences lipid metabolism. Because cholesterol is the substrate from which testosterone is synthesized exposure to these substances has the potential to alter testosterone concentrations. OBJECTIVES: Explore associations of total testosterone and sex hormone-binding globulin (SHBG) concentrations at age 15 with prenatal exposures to PFOS, PFOA, perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) in females. METHODS: Prenatal concentrations of the perfluoroalkyl acids (PFAAs) were measured in serum collected from pregnant mothers at enrollment (1991-1992) in the Avon Longitudinal Study of Parents and Children (ALSPAC). The median gestational age when the maternal blood sample was obtained was 16 weeks (interquartile range: 11- 28 weeks). Total testosterone and SHBG concentrations were measured in serum obtained from their daughters at 15 years of age. Associations between prenatal PFAAs concentrations and reproductive outcomes were estimated using linear regression models (n=72). RESULTS: Adjusted total testosterone concentrations were on average 0.18 nmol/L (95% CI: 0.01, 0.35) higher in daughters with prenatal PFOS in the upper concentration tertile compared to daughters with prenatal PFOS in the lower tertile. Adjusted total testosterone concentrations were also higher in daughters with prenatal concentrations of PFOA (beta = 0.24; 95% CI: 0.05, 0.43) and PFHxS (beta = 0.18; 95% CI: 0.00, 0.35) in the upper tertile compared

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

to daughters with concentrations in the lower tertile. We did not find evidence of associations between PFNA and total testosterone or between any of the PFAAs and SHBG. CONCLUSIONS: Our findings were based on a small study sample and should be interpreted with caution. However, they suggest that prenatal exposure to some PFAAs may alter testosterone concentrations in females.

[*]The Association of Prenatal Exposure to Perfluorinated Chemicals with Maternal Essential and Long-Chain Polyunsaturated Fatty Acids during Pregnancy and the Birth Weight of their Offspring: The Hokkaido Study.
Kishi R., Nakajima T., Goudarzi H., Kobayashi S., Sasaki S., Okada E., Miyashita C., Itoh S., Araki A., Ikeno T., Iwasaki Y. and Nakazawa H.
Environ Health Perspect. 2015 Apr 3. [Epub ahead of print].

BACKGROUND: Fatty acids (FAs) are essential for fetal growth. Exposure to perfluorinated chemicals (PFCs) may disrupt FA homeostasis, but there is no epidemiological data regarding associations of PFCs and FA concentrations. OBJECTIVES: We estimated associations between perfluorooctane sulfonate (PFOS)/perfluorooctanoate (PFOA) concentrations and maternal levels of FAs and triglyceride (TG) and birth size of the offspring. METHODS: 306 mother-child pairs were analyzed in this birth cohort between 2002 and 2005 in Japan. The prenatal PFOS and PFOA levels were measured in maternal serum samples by liquid chromatography-tandem mass spectrometry. Maternal blood levels of 9 FAs and TG were measured by gas chromatography-mass spectrometry and TG-IE kits, respectively. Information of infants' birth size were obtained from participant medical records. RESULTS: The median PFOS and PFOA levels were 5.6 and 1.4 ng/mL, respectively. In the fully adjusted model, including maternal age, parity, annual household income, blood sampling period, alcohol consumption and smoking during pregnancy, PFOS, not PFOA, had a negative association with the levels of palmitic, palmitoleic, oleic, linoleic, alpha-linolenic, and arachidonic acids ($p < 0.005$) and TG (p value=0.016). Females weighed 186.6 g less in mothers whose PFOS levels were in the fourth quartile compared to the first quartile (95% CI: -363.4, -9.8). We observed no significant association between maternal levels of PFOS and birth weight of male infants. CONCLUSIONS: Our data suggest an inverse association between PFOS exposure and polyunsaturated FA levels in pregnant women. We also found a negative association between maternal PFOS levels and female birth weight.

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

***Anthropometry in 5- to 9-Year-Old Greenlandic and Ukrainian Children in Relation to Prenatal Exposure to Perfluorinated Alkyl Substances**.
Hoyer B. B., Ramlau-Hansen C. H., Vrijheid M., Valvi D., Pedersen H. S., Zviezdai V., Jonsson B. A., Lindh C. H., Bonde J. P. and Toft G.
Environ Health Perspect. 2015 123(8):841-6.

BACKGROUND: In some animal studies, perfluorinated alkyl substances are suggested to induce weight gain. Human epidemiological studies investigating these associations are sparse. OBJECTIVE: To examine pregnancy serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and the prevalence of offspring overweight (> 1 standard deviation) and waist-to-height ratio (WHtR) > 0.5 at five to nine years of age. METHODS: Sera from 1,022 pregnant women enrolled in the INUENDO cohort (2002-2004) from Greenland and Kharkiv (Ukraine) were analysed for PFOA and PFOS using liquid chromatography-tandem-mass-spectrometry. Relative risks (RR) of being overweight and having WHtR > 0.5 in relation to continuous and categorised (tertiles) PFOA and PFOS were calculated at follow-up (2010-2012) using generalised linear models. RESULTS: Pooled PFOA median (range) was 1.3 (0.2-5.1) and PFOS median (range) was 10.8 (0.8-73.0) ng/mL. For each natural logarithm-unit (ln-unit) increase of pregnancy PFOA, the adjusted RR of offspring overweight was 1.11 (95% confidence interval (CI): 0.82, 1.53) in Greenlandic children. In Ukrainian children, the adjusted RR of offspring overweight was 1.02 (95% CI: 0.72, 1.44) for each ln-unit increase of pregnancy PFOA. Prenatal exposure to PFOS was not associated with overweight in country-specific or pooled analysis. The adjusted RR of having WHtR > 0.5 for each ln-unit increase of prenatal exposure to PFOA was 1.30 (95% CI: 0.97, 1.74) in the pooled analysis. For one ln-unit increase of prenatal exposure to PFOS, the adjusted RR of having a WHtR > 0.5 was 1.38 (95% CI: 1.05, 1.82) in the pooled analysis. CONCLUSIONS: The results indicate that prenatal PFOA and PFOS exposure may be associated with child waist-to-height ratio > 0.5. Prenatal PFOA and PFOS exposure were not associated with overweight.

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women.
Barrett E. S., Chen C., Thurston S. W., Haug L. S., Sabaredzovic A., Fjeldheim F. N., Frydenberg H., Lipson S. F., Ellison P. T. and Thune I.
Fertil Steril. 2015;103(5):1261-70 e3.

OBJECTIVE: To examine associations between environmental exposure to perfluoroalkyl substances (PFASs) and ovarian hormone concentrations in naturally cycling women. DESIGN: E2 and P were measured in saliva samples collected daily for a single menstrual cycle and concentrations of PFASs (including perfluoroctane sulfonate [PFOS] and perfluorooctanoic acid) were measured in serum samples collected during the same cycle. SETTING: Not applicable. PATIENT(S): A total of 178 healthy, naturally cycling women, aged 25-35 years. INTERVENTION(S): None. MAIN OUTCOME MEASURE(S): Mean follicular E2 (cycle days -7 to -1, where 0 is the day of ovulation); mean luteal P (cycle days +2 to 10). RESULT(S): Among nulliparous, but not parous women, PFOS concentrations were inversely associated with E2 (beta = -0.025, 95% CI -0.043, -0.007) and P (beta = -0.027, 95% CI -0.048, -0.007). Similar, but weaker results were observed for perfluorooctanesulfonic acid. No associations were observed between other PFASs (including perfluoroctanoic acid) and ovarian steroid concentrations, nor were any associations noted in parous women. CONCLUSION(S): Our results demonstrate that PFOS and perfluorooctanesulfonic acid may be associated with decreased production of E2 and P in reproductive age women. These results suggest a possible mechanism by which PFASs affect women's health, and underscore the importance of parity in research on PFASs and women's reproductive health.

***Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years--a prospective study**.
Hoyer B. B., Ramlau-Hansen C. H., Obel C., Pedersen H. S., Hernik A., Ogniev V., Jonsson B. A., Lindh C. H., Rylander L., Rignell-Hydbom A., Bonde J. P. and Toft G.
Environ Health. 2015;14:2.

BACKGROUND: In animal studies, perfluorinated alkyl substances affect growth and neuro-behavioural outcomes. Human epidemiological studies are sparse. The aim was to investigate the association between pregnancy serum concentrations of

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perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) and offspring behaviour and motor development at 5-9 years of age. METHODS: Maternal sera from the INUENDO cohort (2002-2004) comprising 1,106 mother-child pairs from Greenland, Kharkiv (Ukraine) and Warsaw (Poland) were analysed for PFOS and PFOA, using liquid-chromatography-tandem-mass-spectrometry. Exposures were grouped into country specific as well as pooled tertiles as well as being used as continuous variables for statistical analyses. Child motor development and behaviour at follow-up (2010-2012) were measured by the Developmental Coordination Disorder Questionnaire 2007 (DCDQ) and Strength and Difficulties Questionnaire (SDQ), respectively. Exposure-outcome associations were analysed by multiple logistic and linear regression analyses. RESULTS: In the pooled analysis, odds ratio (OR) (95% confidence interval (CI)) for hyperactivity was 3.1 (1.3, 7.2) comparing children prenatally exposed to the highest PFOA tertile with those exposed to the lowest PFOA tertile. Comparing children in the highest PFOS tertile with those in the lowest PFOS tertile showed elevated but statistically non-significant OR of hyperactivity (OR (95% CI) 1.7 (0.9, 3.2)). In Greenland, elevated PFOS was associated with higher SDQ-total scores indicating more behavioural problems (beta (95% CI)=1.0 (0.1, 2.0)) and elevated PFOA was associated with higher hyperactivity sub-scale scores indicating more hyperactive behaviour (beta (95% CI)=0.5 (0.1, 0.9)). Prenatal PFOS and PFOA exposures were not associated with motor difficulties. CONCLUSIONS: Prenatal exposure to PFOS and PFOA may have a small to moderate effect on children's neuro-behavioural development, specifically in terms of hyperactive behaviour. The associations were strongest in Greenland where exposure contrast is largest.

***PFOA and PFOS serum levels and miscarriage risk**.
Darrow L. A., Howards P. P., Winquist A. and Steenland K.
Epidemiology. 2014;25(4):505-12.

BACKGROUND: Serum concentrations of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were assessed in relation to miscarriage in a population of mid-Ohio River Valley residents highly exposed to PFOA through contaminated drinking water. METHODS: Serum PFOA and PFOS concentrations were measured in 1129 women in 2005-2006 who reported pregnancy outcomes in follow-up interviews between 2008 and 2011. In the analysis, we included 1438 reported live births, stillbirths, and miscarriages with estimated conception dates after the serum measurements. Preconception serum levels of PFOA and PFOS were analyzed in

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relation to miscarriage using logistic regression and generalized estimating equations. RESULTS: There was little evidence of association between PFOA and miscarriage. For PFOS, when including all reported prospective pregnancies, the odds ratio of miscarriage per log ng/ml increase was 1.21 (95% confidence interval = 0.94-1.55); in subanalyses restricted to each woman's first pregnancy conceived after the serum measurement, the odds ratio was 1.34 (1.02-1.76). Categorical analyses showed elevated odds ratios for the top 4 quintiles relative to the first quintile, without a monotonic trend. Positive associations between PFOS and miscarriage were strongest among nulligravid pregnancies. CONCLUSIONS: In this prospective study of miscarriage in a population exposed to high levels of PFOA and background levels of PFOS, we found little evidence of association with serum levels of PFOA and limited evidence of association with serum levels of PFOS.

Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study.
Webster G. M., Venners S. A., Mattman A. and Martin J. W.
Environ Res. 2014;133:338-47.

BACKGROUND: Associations between perfluoroalkyl acids (PFASs) and human thyroid hormone levels remain unclear, especially during early pregnancy when small changes in maternal thyroid hormones can affect fetal brain development. OBJECTIVES: To examine associations between maternal serum PFAS levels and maternal thyroid hormone levels in the early 2nd trimester of pregnancy. METHODS: Participants were euthyroid pregnant women (n=152) enrolled in the Chemicals, Health and Pregnancy (CHirP) study based in Vancouver, Canada. Associations between maternal serum PFASs, including perfluorohexanesulfonate (PFHxS), perfluorononanoate (PFNA), perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and repeated measures of maternal thyroid hormones, including free thyroxine (fT4), total thyroxine (TT4) and thyroid stimulating home (TSH) were examined using mixed effects linear models. Associations were considered in all women, then separately in women with high (≥ 9 IU/mL) vs normal (<9 IU/mL) levels of thyroid peroxidase antibody (TPOAb), a marker of autoimmune hypothyroidism (Hashimoto's disease). RESULTS: Median PFAS concentrations (ng/mL) in maternal sera were 1.0 (PFHxS), 0.6 (PFNA), 1.7 (PFOA) and 4.8 (PFOS). PFASs were not associated with fT4, TT4 or TSH among women with normal TPOAb. However, among the 9% of women with high TPOAb (n=14), interquartile range (IQR) increases of PFASs were associated with a 46-69% increase in maternal TSH (95% CIs ranging from 8% to 123%) (PFNA, PFOA and PFOS only), and with a 3% to 7% decrease in maternal fT4 (95% CIs ranging from -18% to 5%) (all 4 PFASs). PFNA was also associated with higher maternal TSH in the

whole sample. CONCLUSIONS: PFASs were positively associated with TSH, and weakly negatively associated with fT4 in the subset of pregnant women with high TPOAb, which occurs in 6-10% of pregnancies. PFASs may exacerbate the already high TSH and low fT4 levels in these women during early pregnancy, which is a critical time of thyroid hormone-mediated fetal brain development. The clinical significance of these findings is not clear. We propose a "multiple hit hypothesis" to explain these findings; this hypothesis deserves evaluation in larger, more representative study samples.

***Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children**.
Liew Z., Ritz B., Bonefeld-Jorgensen E. C., Henriksen T. B., Nohr E. A., Bech B. H., Fei C., Bossi R., von E. O. S., Streja E., Uldall P. and Olsen J.
Am J Epidemioly. 2014;180(6):574-81.

Perfluoroalkyl substances (PFASs) are persistent pollutants and endocrine disruptors that may affect fetal brain development. We investigated whether prenatal exposure to PFASs increases the risk of congenital cerebral palsy (CP). The source population for this study includes 83,389 liveborn singletons and mothers enrolled in the Danish National Birth Cohort during 1996-2002. We identified 156 CP cases by linking the cohort to the Danish National Cerebral Palsy Register, and we randomly selected 550 controls using a case-cohort design. We measured 16 PFASs in maternal plasma collected in early or midpregnancy, and 6 PFASs were quantifiable in more than 90% of the samples. We found a higher risk of CP in boys with higher maternal PFAS levels; per 1-unit (natural-log ng/mL) increase, the risk ratios were 1.7 (95% confidence interval: 1.0, 2.8) for perfluorooctane sulfonate and 2.1 (95% confidence interval: 1.2, 3.6) for perfluorooctanoic acid. We also observed a dose-response pattern of CP risk in boys per quartile of maternal level of perfluorooctane sulfonate and perfluorooctanoic acid (P for trend < 0.01). PFASs were associated with both unilateral and bilateral spastic CP subphenotypes. No association between PFASs and CP was found in girls. Prenatal exposures to PFASs may increase the risk of CP in boys, but the finding is novel and replication is needed.

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Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study.
Vagi S. J., Azziz-Baumgartner E., Sjodin A., Calafat A. M., Dumesic D., Gonzalez L., Kato K., Silva M. J., Ye X. and Azziz R.
BMC Endocr Disord. 2014;14:86.

BACKGROUND: Polycystic Ovary Syndrome (PCOS) is an endocrine-metabolic disorder that affects approximately 6-10% of women of child-bearing age. Although preliminary studies suggest that certain pollutants may act as endocrine disruptors in animals, little is known about their potential association with PCOS. The objective of this case-control pilot study is to determine whether women with PCOS have higher concentrations of specific environmental contaminants compared to women who have not developed PCOS. METHODS: Fifty-two PCOS case-patients (diagnosed using the National Institutes of Health 1990 definition) and 50 controls were recruited in 2007-2008, from an urban academic medical center in Los Angeles, CA. Brominated diphenyl ethers, polychlorinated biphenyls (PCBs), organochlorine pesticides, and perfluorinated compounds (PFCs) were measured in serum, and phthalates metabolites and bisphenol A (BPA) in urine. RESULTS: PCOS case-patients had significantly higher geometric mean (GM) serum concentrations of two PFCs: perfluorooctanoate (PFOA) (GMcases = 4.1 mug/L, GMcontrols = 2.3 mug/L; p = 0.001) and perfluorooctane sulfonate (PFOS) (GMcases = 8.2 mug/L, GMcontrols = 4.9 mug/L; p = 0.01), and lower urinary concentrations of monobenzyl phthalate (mBzP) (GMcases = 7.5 mug/g creatinine, GMcontrols = 11.7 mug/g creatinine; p = 0.02). Logistic regression, controlling for body mass index, age and race, identified an increased likelihood of PCOS in subjects with higher serum concentrations of PFOA and PFOS (adjusted-ORs = 5.8-6.9, $p < 0.05$), and with lower urine concentrations of mBzP and mono-n-butyl phthalate (mBP) (aORs = 0.14-0.25, $p < 0.05$). CONCLUSIONS: Our data suggest that PCOS case-patients may differ from controls in their environmental contaminant profile. PCOS subjects had higher serum concentrations of two PFCs, PFOA and PFOS, and lower urine concentrations of mBP and mBzP. Future studies are needed to confirm these preliminary findings and determine if these chemicals or their precursors may have a role in the pathogenesis of PCOS.

***Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age**.

Chen M. H., Ha E. H., Liao H. F., Jeng S. F., Su Y. N., Wen T. W., Lien G. W., Chen C. Y., Hsieh W. S. and Chen P. C.

Epidemiology. 2013;24(6):800-8.

BACKGROUND: Epidemiologic data regarding the potential neurotoxicity of perfluorinated compounds (PFCs) are inconclusive. We investigated the associations between in utero exposure to perfluorooctanoic acid (PFOA) and perfluorooctyl sulfonate (PFOS) and early childhood neurodevelopment. METHODS: We recruited 239 mother-infant pairs in northern Taiwan from the Taiwan Birth Panel Study, which was established in 2004. We examined the association between PFCs in cord blood and children's neurodevelopment at 2 years of age, using the Comprehensive Developmental Inventory for Infants and Toddlers. This tool contains cognitive, language, motor, social, and self-help domains; test scores were further transformed into developmental quotients according to standardized norms. All multivariate regression models were adjusted for infant sex and gestational age, maternal education, family income, cord blood cotinine levels, postnatal environmental tobacco smoke exposure, and breastfeeding. RESULTS: Prenatal PFOS concentrations in both untransformed and natural log (Ln)-transformed values were associated with adverse performance on the whole test and the domains related to development. A dose-response relationship was observed when PFOS levels were categorized into four groups. This association was most obvious in relation to the gross-motor subdomain. Across the PFOS interquartile range, the quotients of the gross-motor subdomain decreased by 3.7 points (95% confidence interval [CI] = -6.0 to -1.5), with an increasing odds ratio of poor performance (2.4; 95% CI = 1.3 to 4.2). In contrast, measures of association between PFOA concentrations and test scores were close to null. CONCLUSIONS: Prenatal exposure to PFOS, but not PFOA, may affect children's development, especially gross-motor development at 2 years of age.

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***Serum perfluorooctanoic acid and perfluorooctane sulfonate concentrations in relation to birth outcomes in the Mid-Ohio Valley, 2005-2010.**
Darrow L. A., Stein C. R. and Steenland K.
Environ Health Perspect. 2013;121(10):1207-13.

BACKGROUND: Previous research suggests perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) may be associated with adverse pregnancy outcomes. OBJECTIVE: We conducted a population-based study of PFOA and PFOS and birth outcomes from 2005 through 2010 in a Mid-Ohio Valley community exposed to high levels of PFOA through drinking-water contamination. METHODS: Women provided serum for PFOA and PFOS measurement in 2005-2006 and reported reproductive histories in subsequent follow-up interviews. Reported singleton live births among 1,330 women after 1 January 2005 were linked to birth records (n = 1,630) to identify the outcomes of preterm birth (< 37 weeks gestation), pregnancy-induced hypertension, low birth weight (< 2,500 g), and birth weight (grams) among full-term infants. RESULTS: We observed little or no evidence of association between maternal serum PFOA or PFOS and preterm birth (n = 158) or low birth weight (n = 88). Serum PFOA and PFOS were both positively associated with pregnancy-induced hypertension (n = 106), with adjusted odds ratios (ORs) per log unit increase in PFOA and PFOS of 1.27 (95% CI: 1.05, 1.55) and 1.47 (95% CI: 1.06, 2.04), respectively, but associations did not increase monotonically when categorized by quintiles. Results of subanalyses restricted to pregnancies conceived after blood collection were consistent with the main analyses. There was suggestion of a modest negative association between PFOS and birth weight in full-term infants (-29 g per log unit increase; 95% CI: -66, 7), which became stronger when restricted to births conceived after the blood sample collection (-49 g per log unit increase; 95% CI: -90, -8). CONCLUSION: Results provide some evidence of positive associations between measured serum perfluorinated compounds and pregnancy-induced hypertension and a negative association between PFOS and birth weight among full-term infants.

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Association between perfluoroalkyl substances and thyroid stimulating hormone among pregnant women: a cross-sectional study.

Wang Y., Starling A. P., Haug L. S., Eggesbo M., Becher G., Thomsen C., Travlos G., King D., Hoppin J. A., Rogan W. J. and Longnecker M. P.
Environ Health. 2013;12(1):76.

BACKGROUND: Perfluoroalkyl substances (PFASs) are a group of highly persistent chemicals that are widespread contaminants in wildlife and humans. Exposure to PFAS affects thyroid homeostasis in experimental animals and possibly in humans. The objective of this study was to examine the association between plasma concentrations of PFASs and thyroid stimulating hormone (TSH) among pregnant women. METHODS: A total of 903 pregnant women who enrolled in the Norwegian Mother and Child Cohort Study from 2003 to 2004 were studied. Concentrations of thirteen PFASs and TSH were measured in plasma samples collected around the 18th week of gestation. Linear regression models were used to evaluate associations between PFASs and TSH. RESULTS: Among the thirteen PFASs, seven were detected in more than 60% of samples and perfluorooctane sulfonate (PFOS) had the highest concentrations (median, 12.8 ng/mL; inter-quartile range [IQR], 10.1 -16.5 ng/mL). The median TSH concentration was 3.5 (IQR, 2.4 - 4.8) μIU/mL. Pregnant women with higher PFOS had higher TSH levels. After adjustment, with each 1 ng/mL increase in PFOS concentration, there was a 0.8% (95% confidence interval: 0.1%, 1.6%) rise in TSH. The odds ratio of having an abnormally high TSH, however, was not increased, and other PFASs were unrelated to TSH. CONCLUSIONS: Our results suggest an association between PFOS and TSH in pregnant women that is small and may be of no clinical significance.

Neonatal-maternal factors and perfluoroalkyl substances in cord blood.
Lien G. W., Huang C. C., Wu K. Y., Chen M. H., Lin C. Y., Chen C. Y., Hsieh W. S. and Chen P. C.
Chemosphere. 2013;92(7):843-50.

Perfluoroalkyl substances (PFASs) can cross the placenta, enter fetal circulation, and were found to correlate with adverse fetal growth. However, determinants of cord blood PFASs are not fully characterized. The study aimed to explore the association between PFASs and neonatal-maternal factors within a Taiwanese birth cohort. We selected subjects from Taiwan Birth Panel Study, which enrolled 486 infant-mother pairs in 2004-2005. We collected cord blood and analyzed perfluorooctanoic acid (PFOA), perfluorooctanyl sulfonate (PFOS), perfluorononanoic acid (PFNA) and

perfluoroundecanoic acid (PFUA) using a simple protein precipitation and an ultra-high performance liquid chromatography/tandem mass spectrometry. We retrieved information pertaining to maternal socio-demographics, lifestyle- and dietary-related factors through structured questionnaires during the postpartum hospital stay. A total of 439 subjects, with 90% response rate, have completed serum analysis and questionnaire survey. The median concentrations for PFOA, PFOS, PFNA, and PFUA in cord blood were 1.86, 5.67, 3.00, and 13.5 ngmL(-1), respectively. After adjusting for potential confounders, multiple linear regression models revealed that log10-PFOA was positively associated with maternal age (beta=0.011) and negatively associated with multiparity (beta=-0.044). Log10-PFOS was negatively correlated with birth weight (beta=-0.011) and higher maternal education (senior high school: beta=-0.067; university: beta=-0.088). Log10-PFUA tended to negatively associate with gender, male infants (beta=-0.075), and using cosmetics during pregnancy (beta=-0.065). Interestingly, presence of cockroaches in the home was positively associated with log10-PFOA (beta=0.041) and log10-PFNA (beta=0.123). In conclusion, this study demonstrated several factors to correlate with cord blood PFASs and further investigation are still needed for confirmation of exposure routes.

***Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood**.
Granum B., Haug L. S., Namork E., Stolevik S. B., Thomsen C., Aaberge I. S., van Loveren H., Lovik M. and Nygaard U. C.
J Immunotoxicol. 2013;10(4):373-9.

Perfluoroalkyl substances (PFAS) are suggested to have immunosuppressive effects; exposure in utero and in the first years of life is of special concern as fetuses and small children are highly vulnerable to toxicant exposure. The objective of this study was to investigate the effect of pre-natal exposure to PFAS on responses to pediatric vaccines and immune-related health outcomes in children up to 3 years of age. In the prospective birth-cohort BraMat, a sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa), pregnant women from Oslo and Akershus, Norway, were recruited during 2007-2008. Three annual questionnaire-based follow-ups were performed. Blood samples were collected from the mothers at the time of delivery and from the children at the age of 3 years. As a measure of pre-natal exposure to PFAS, the concentrations of perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were determined in maternal blood

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from 99 BraMat participants. Main outcome measures were anti-vaccine antibody levels, common infectious diseases and allergy- and asthma-related health outcomes in the children up to the age of 3 years. There was an inverse association between the level of anti-rubella antibodies in the children's serum at age 3 years and the concentrations of the four PFAS. Furthermore, there was a positive association between the maternal concentrations of PFOA and PFNA and the number of episodes of common cold for the children, and between PFOA and PFHxS and the number of episodes of gastroenteritis. No associations were found between maternal PFAS concentrations and the allergy- and asthma-related health outcomes investigated. The results indicate that pre-natal exposure to PFAS may be associated with immunosuppression in early childhood.

PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men.
Joensen U. N., Veyrand B., Antignac J. P., Blomberg Jensen M., Petersen J. H., Marchand P., Skakkebaek N. E., Andersson A. M., Le Bizec B. and Jorgensen N.
Hum Reprod. 2013;28(3):599-608.

STUDY QUESTION: Is exposure to perfluorinated compounds (PFCs) associated with testicular function (reproductive hormone levels and semen quality) in healthy men? SUMMARY ANSWER: PFOS levels were significantly negatively associated with serum testosterone (total and calculated free), but not with any other reproductive hormones or semen quality. WHAT IS KNOWN ALREADY: In animals, some PFCs have endocrine disrupting potential, but few studies have investigated PFCs in relation to human testicular function. Previously, we and others have observed a negative association between serum PFC levels and sperm morphology. The potential associations with reproductive hormones remain largely unresolved. STUDY DESIGN, SIZE, DURATION: A cross-sectional study of 247 men was conducted during 2008-2009. PARTICIPANTS/MATERIALS, SETTING, METHODS: Healthy men from the general population, median age of 19 years, gave serum and semen samples. Serum samples were analysed for total testosterone (T), estradiol (E), sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH) and inhibin-B and 14 PFCs, including perfluorooctanesulfonate (PFOS). Semen samples were analysed according to the WHO criteria. MAIN RESULTS AND THE ROLE OF CHANCE: PFOS levels were negatively associated with testosterone (T), calculated free testosterone (FT), free androgen index (FAI) and ratios of T/LH, FAI/LH and FT/LH. Other PFCs were found at lower levels than PFOS and did not exhibit the same associations. PFC levels were not significantly associated with semen quality. PFOS levels in these samples collected in 2008-2009 were lower than in our previous study of men

participating in 2003. LIMITATIONS, REASONS FOR CAUTION: Results were robust to adjustment for relevant confounders; however, the possibility of chance associations due to multiple testing or effects of uncontrolled confounding cannot be ruled out. WIDER IMPLICATIONS OF THE FINDINGS: Our previous findings of decreased sperm morphology in the most highly PFC exposed men were not replicated, possibly due to a lack of highly exposed individuals; however, a recent independent study also did corroborate such an inverse association. The negative association between serum PFOS and testosterone indicates that testosterone production may be compromised in individuals with high PFOS exposure. STUDY FUNDING/COMPETING INTEREST(S): The authors received financial support from the European Commission (DEER, FP7-2007-212844), the Danish Agency for Science, Technology and Innovation (grant nos. 27107068 and 09-067180), Rigshospitalet (grant no. 961506336), the University of Copenhagen, the Danish Ministry of Health and the Danish Environmental Protection Agency (MST-621-00013), and Kirsten and Freddy Johansen Foundation (grant no. 95-103-72087). The funding organizations played no role in the design and conduct of the study, in collection, management, analysis and interpretation of the data; or in the presentation, review or approval of the manuscript. The authors declare that they have no competing financial interests.

Concentrations of perfluoroalkyl compounds in maternal and umbilical cord sera and birth outcomes in Korea.
Lee Y. J., Kim M. K., Bae J. and Yang J. H.
Chemosphere. 2013;90(5):1603-9.

This study analyzed the concentrations of perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) in maternal and umbilical cord sera at delivery from the general population in Korea. Seventy samples were analyzed with ion-pairing and LC/MS/MS. PFOS, PFOA and PFHxS were detected in both maternal and umbilical cord sera. There was a high correlation of PFC concentrations between maternal and cord serum samples, implying transplacental transport. Ranking of transplacental transfer efficiency was PFOA>PFHxS>PFOS. Student's t-tests revealed that concentrations of maternal PFOA were related with decreases in birth weight, birth length and ponderal index, suggesting a possible impact on fetal growth. With multiple logistic regression models, maternal PFOS concentration showed a significant inverse association with ponderal index (OR=0.22; 95% CI, 0.05-0.90). Umbilical cord PFHxS concentration showed a significant inverse association with birth weight (OR=0.26; 95% CI, 0.08-0.85) or a marginally significant inverse association with birth length (OR=0.33; 95% CI, 0.09-1.17). This is the first report demonstrating an inverse association of birth outcomes with PFHxS exposure.

Concentrations of maternal PFOA were decreased with parity, implying that delivery is one of the major routes for PFOA elimination in women. This study demonstrated prenatal exposure of PFCs through placental transfer which could result in possible developmental effects in the population sampled. Our results may provide data basis to conduct a larger scale investigation into developmental effects of PFCs in the future and contribute to understanding levels of PFC contaminations from a variety of populations in the globe.

***Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls**.
Maisonet M., Terrell M. L., McGeehin M. A., Christensen K. Y., Holmes A., Calafat A. M. and Marcus M.
Environ Health Perspect. 2012;120(10):1432-7.

BACKGROUND: Prenatal exposures to polyfluoroalkyl compounds (PFCs) may be associated with adverse changes in fetal and postnatal growth. OBJECTIVE: We explored associations of prenatal serum concentrations of perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexane sulfonate (PFHxS) with fetal and postnatal growth in girls. METHODS: We studied a sample of 447 singleton girls and their mothers participating in the Avon Longitudinal Study of Parents and Children (ALSPAC). Data on weight and length were obtained at birth and at 2, 9, and 20 months. Serum samples were obtained in 1991-1992, from mothers during pregnancy. We explored associations between prenatal PFC concentrations and weight at birth as well as longitudinal changes in weight-for-age SD scores between birth and 20 months. RESULTS: PFOS (median, 19.6 ng/mL), PFOA (median, 3.7 ng/mL), and PFHxS (median, 1.6 ng/mL) were detected in 100% of samples. On average, girls born to mothers with prenatal concentrations of PFOS in the upper tertile weighed 140 g less [95% confidence interval (CI): -238, -42] at birth than girls born to mothers with concentrations in the lower tertile in adjusted models. Similar patterns were seen for PFOA (-133 g; 95% CI: -237, -30) and PFHxS (-108 g; 95% CI: -206, -10). At 20 months, however, girls born to mothers with prenatal concentrations of PFOS in the upper tertile weighed 580 g more (95% CI: 301, 858) when compared with those in the lower tertile. No differences in weight were found for PFOA and PFHxS. CONCLUSIONS: Girls with higher prenatal exposure to each of the PFCs examined were smaller at birth than those with lower exposure. In addition, those with higher exposure to PFOS were larger at 20 months.

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Perfluorinated compounds in umbilical cord blood and adverse birth outcomes.
Chen M. H., Ha E. H., Wen T. W., Su Y. N., Lien G. W., Chen C. Y., Chen P. C. and Hsieh W. S.
PLoS One. 2012;7(8):e42474.

BACKGROUND: Previous animal studies have shown that perfluorinated compounds (PFCs) have adverse impacts on birth outcomes, but the results have been inconclusive in humans. We investigated associations between prenatal exposure to perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluoroundecanoic acid (PFUA) and birth outcomes. METHODS: In total, 429 mother-infant pairs were recruited from the Taiwan Birth Panel Study (TBPS). Demographic data were obtained by interviewing mothers using a structured questionnaire and birth outcomes were extracted from medical records. Cord blood was collected for PFOA, PFOS, PFNA, and PFUA analysis by ultra-high-performance liquid chromatography/tandem mass spectrometry. RESULTS: The geometric mean (standard deviation) levels of PFOA, PFOS, PFNA, and PFUA in cord blood plasma were 1.84 (2.23), 5.94 (1.95), 2.36(4.74), and 10.26 (3.07) ng/mL, respectively. Only PFOS levels were found to be inversely associated with gestational age, birth weight, and head circumference [per ln unit: adjusted beta (95% confidence interval, CI) = -0.37 (-0.60, -0.13) wks, -110.2 (-176.0, -44.5) gm and -0.25 (-0.46, -0.05) cm]. Additionally, the odds ratio of preterm birth, low birth weight, and small for gestational age increased with PFOS exposure [per ln unit: adjusted odds ratio (OR) (95%CI) = 2.45 (1.47, 4.08), 2.61(0.85, 8.03) and 2.27 (1.25, 4.15)]. When PFOS levels were divided into quartiles, a dose-response relation was observed. However, PFOA, PFNA, and PFUA were not observed to have any convincing impact on birth outcomes. CONCLUSIONS: An adverse dose-dependent association was observed between prenatal PFOS exposure and birth outcomes. However, no associations were found for the other examined PFCs.

Exposure to perfluorinated compounds and human semen quality in Arctic and European populations.
Toft G., Jonsson B. A., Lindh C. H., Giwercman A., Spano M., Heederik D., Lenters V., Vermeulen R., Rylander L., Pedersen H. S., Ludwicki J. K., Zviezdai V. and Bonde J. P.
Hum Reprod. 2012;27(8):2532-40.

BACKGROUND: Perfluorinated compounds (PFCs) have been suspected to adversely affect human reproductive health. The aim of this study was to investigate the associations between PFC exposure and male semen quality. METHODS: PFCs were measured in serum from 588 partners of pregnant women from Greenland, Poland and Ukraine who provided a semen sample, using liquid chromatography tandem mass spectrometry. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) could be detected in >97% of the samples. The associations between levels of these compounds and semen volume, sperm concentration, total sperm count, motility and morphology were assessed. RESULTS: Across countries, sperm concentration, total sperm count and semen volume were not consistently associated with PFOS, PFOA, PFHxS or PFNA levels. The proportion of morphologically normal cells was 35% lower [95% confidence interval (CI): 4-66%) for the third tertile of PFOS exposure as compared with the first. A similar reduction was found in relation to increasing PFHxS levels. At the third PFOA exposure tertile, the percentage of motile spermatozoa was 19% (95% CI: 1 to 39%) higher than in the first. CONCLUSIONS: The most robust finding in the present study was the negative associations between PFOS exposure and sperm morphology suggesting adverse effects of PFOS on semen quality, possibly due to interference with the endocrine activity or sperm membrane function. It cannot be excluded that this association and the positive association between PFOA and semen motility, which was not consistent across countries, might represent a chance finding due to the multiple statistical tests being performed.

Perfluorinated compounds and subfecundity in pregnant women.
Whitworth K. W., Haug L. S., Baird D. D., Becher G., Hoppin J. A., Skjaerven R., Thomsen C., Eggesbo M., Travlos G., Wilson R. and Longnecker M. P.
Epidemiology. 2012;23(2):257-63.

BACKGROUND: Perfluorinated compounds are ubiquitous pollutants; epidemiologic data suggest they may be associated with adverse health outcomes, including subfecundity. We examined subfecundity in relation to 2 perfluorinated compounds-perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). METHODS: This

case-control analysis included 910 women enrolled in the Norwegian Mother and Child Cohort Study in 2003 and 2004. Around gestational week 17, women reported their time to pregnancy and provided blood samples. Cases consisted of 416 women with a time to pregnancy greater than 12 months, considered subfecund. Plasma concentrations of perfluorinated compounds were analyzed using liquid chromatography-mass spectrometry. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each pollutant quartile using logistic regression. Estimates were further stratified by parity. RESULTS: The median plasma concentration of PFOS was 13.0 ng/mL (interquartile range [IQR] = 10.3-16.6 ng/mL) and of PFOA was 2.2 ng/mL (IQR = 1.7-3.0 ng/mL). The relative odds of subfecundity among parous women was 2.1 (95% CI = 1.2-3.8) for the highest PFOS quartile and 2.1 (1.0-4.0) for the highest PFOA quartile. Among nulliparous women, the respective relative odds were 0.7 (0.4-1.3) and 0.5 (0.2-1.2). CONCLUSION: Previous studies suggest that the body burden of perfluorinated compounds decreases during pregnancy and lactation through transfer to the fetus and to breast milk. Afterward, the body burden may increase again. Among parous women, increased body burden may be due to a long interpregnancy interval rather than the cause of a long time to pregnancy. Therefore, data from nulliparous women may be more informative regarding toxic effects of perfluorinated compounds. Our results among nulliparous women did not support an association with subfecundity.

Serum levels of perfluorinated compounds and sperm Y:X chromosome ratio in two European populations and in Inuit from Greenland.
Kvist L., Giwercman Y. L., Jonsson B. A., Lindh C. H., Bonde J. P., Toft G., Strucinski P., Pedersen H. S., Zvyezday V. and Giwercman A.
Reprod Toxicol. 2012;34(4):644-50.

This study investigated whether perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), which exhibit reproductive toxicity in experimental animals, affect sperm sex chromosome ratio. The Y:X ratio was determined by fluorescence in situ hybridization. Serum concentrations of PFOA and PFOS were measured in 607 men from Greenland, Poland and Ukraine using liquid chromatography-tandem mass spectrometry. Data was analyzed by linear and nonlinear regression. We observed no associations between PFOA and Y:X ratio (p=0.845 in a linear model, p=0.296 in a nonlinear model). A positive nonlinear association between PFOS and Y:X ratio was observed (p=0.016), with no association in a linear model (p=0.118). Analyzing the populations separately, a negative trend between categorized PFOS exposure and Y:X ratio was observed for the Inuit (B=-0.002, p=0.044). In conclusion, there was a negative trend between Y:X ratio and PFOS

in the Inuit, while there was no association between PFOA and the Y:X ratio in adult men.

Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones.
Kim S., Choi K., Ji K., Seo J., Kho Y., Park J., Kim S., Park S., Hwang I., Jeon J., Yang H. and Giesy J. P.
Environ Sci Technol. 2011;45(17):7465-72.

While the results of animal studies have shown that perfluorinated compounds (PFCs) can modulate concentrations of thyroid hormones in blood, limited information is available on relationships between concentrations of PFCs in human blood serum and fetal thyroid hormones. The relationship between concentrations of PFCs in blood and fetal thyroid hormone concentrations or birth weight, and ratios of major PFCs between maternal and fetal serum were determined. Concentrations of PFCs were measured in blood serum of pregnant women (n = 44), fetal cord blood serum (n = 43) and breast milk (n = 35). Total concentrations of thyroxin (T4), triiodothyronin (T3) and thyroid stimulating hormone (TSH) in blood serum were also quantified. The ratios of major PFCs in maternal versus fetal serum were 1:1.93, 1.02, 0.72, and 0.48 for perfluorotridecanoic acid (PFTrDA), perfluorooctanoic acid (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS), respectively. Fetal PFOS, PFOA, PFTrDA and maternal PFTrDA were correlated with fetal total T4 concentrations, but after adjusting for major covariates, most of the relationships were no longer statistically significant. However, the significant negative correlations between maternal PFOS and fetal T3, and maternal PFTrDA and fetal T4 and T3 remained. Since thyroid hormones are crucial in the early development of the fetus, its clinical implication should be evaluated. Given the observed trans-placental transfer of PFCs, efforts should be also made to elucidate the exposure sources among pregnant women.

***The effect of prenatal perfluorinated chemicals exposures on pediatric atopy**.
Wang I. J., Hsieh W. S., Chen C. Y., Fletcher T., Lien G. W., Chiang H. L., Chiang C. F., Wu T. N. and Chen P. C.
Environ Res. 2011;111(6):785-91.

BACKGROUND: The role of perfluorinated compounds (PFCs) in the immune system and allergic diseases is not well-known. This study examined the effects of pre-natal

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

exposure to PFCs on immunoglobulin E (IgE) levels and atopic dermatitis (AD). METHODS: In Taiwan Birth Panel cohort study, newborns with cord blood and peri-natal factors (i.e. birth body weight, weeks of gestation, and type of delivery) gathered at birth were evaluated. At the age of 2 years, information on the development of AD, environmental exposures, and serum total IgE were collected. The AD and non-AD children were compared for the concentration of cord blood serum PFCs measured by Ultra-performance liquid chromatography/triple-quadrupole mass (UPLC-MS/MS). Correlations among cord blood IgE, serum total IgE at 2 years of age, and cord blood PFC levels were made. RESULTS: Of 244 children who completed the follow-up and specimen collections, 43 (17.6%) developed AD. Concentrations of cord blood serum perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were median (range) 1.71 (0.75-17.40), 5.50 (0.11-48.36), 2.30 (0.38-63.87), and 0.035 (0.035-0.420)ng/mL, respectively. PFOA and PFOS levels positively correlated with cord blood IgE levels (per ln-unit: beta=0.134 KU/l, p=0.047 for PFOA; beta=0.161 KU/l, p=0.017 for PFOS). Analyses stratified by gender revealed that PFOA and PFOS levels positively correlated with cord blood IgE levels only in boys (per ln-unit: beta=0.206 KU/l, p=0.025 for PFOA; beta=0.175 KU/l, p=0.053 for PFOS). When dividing cord blood serum PFCs into quartiles in the fully adjusted models, AD had no significant association with PFOS. CONCLUSIONS: Pre-natal PFOA and PFOS exposures positively correlated with cord blood IgE levels.

Perfluorocarbon exposure, gender and thyroid function in the C8 Health Project.
Knox S. S., Jackson T., Frisbee S. J., Javins B. and Ducatman A. M.
J Toxicol Sci. 2011;36(4):403-10.

Perfluorocarbons from common household products such as food containers, stain-resistant protection for clothing, furniture and carpets, paints, and fire-fighting foams are found in soil, water, plants, animal and human serum worldwide. Previous research has shown a significant association between these chemicals and thyroid disease in women. The present data from the C8 Health Project assessed thyroid function in a cross-sectional analysis of 52,296 adults with a year or more of exposure to perfluorooctanoate (PFOA) from drinking water. Outcomes were: thyroxine, T3 uptake, and thyroid stimulating hormone (TSH). Analyses were stratified by gender and age group (< 20 - < 50 years and > 50). Both PFOA and perfluorooctane sulfonate (PFOS) were associated with significant elevations in serum thyroxine and a significant reduction in T3 uptake in all participants. There were also significant gender/PFOS interactions for T3 uptake and thyroxine, as well as gender/PFOA interactions for T3

uptake. Results provide evidence for disruption of thyroid function related to these common chemicals and possible mechanisms are discussed.

Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant.
Lopez-Espinosa M. J., Fletcher T., Armstrong B., Genser B., Dhatariya K., Mondal D., Ducatman A. and Leonardi G.
Environ Sci Technol. 2011;45(19):8160-6.

Animal studies suggest that perfluorocarbons (PFCs) may alter sexual maturation. Relationships of human PFC exposure with puberty are not clear. We conducted a cross-sectional study to investigate whether perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were associated with indicators of sexual maturation in a 2005-2006 survey of residents with PFOA water contamination from the Mid-Ohio Valley. Participants were 3076 boys and 2931 girls aged 8-18 years. They were classified as having reached puberty based on either hormone levels (total >50 ng/dL and free >5 pg/mL testosterone in boys and estradiol >20 pg/mL in girls) or onset of menarche. We estimated the odds of having reached puberty classified by these criteria and the fitted median age of reaching puberty in relation to serum PFOA and PFOS concentrations measured when puberty status was assigned. For boys, there was a relationship of reduced odds of reached puberty (raised testosterone) with increasing PFOS (delay of 190 days between the highest and lowest quartile). For girls, higher concentrations of PFOA or PFOS were associated with reduced odds of postmenarche (130 and 138 days of delay, respectively). In conclusion, our study showed a later age of puberty in this population correlated with PFC concentrations.

***Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy**.
Andersen C. S., Fei C., Gamborg M., Nohr E. A., Sorensen T. I. and Olsen J.
Am J Epidemiol. 2010;172(11):1230-7.

Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are persistent chemicals that may affect growth early in life. The authors estimated the associations between maternal plasma levels of PFOS and PFOA and infants' weight, length, and body mass index development during the first year of life. Fourteen hundred women were randomly selected from the Danish National Birth Cohort among those who

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provided blood samples early in pregnancy and gave birth to liveborn singletons between 1996 and 2002. Weight and length information at 5 and 12 months of age was available for 1,010 children. Multiple linear regression models were used for analyses, and maternal PFOS and PFOA concentrations (ng/mL) were inversely related to children's weight in the first year of life: adjusted regression coefficients: −1.1 g (95% confidence interval (CI): −4.6, 2.3) at 5 months and −5.8 g (95% CI: −10.4, −1.2) at 12 months for PFOS; −10.6 g (95% CI: −30.2, 8.9) at 5 months and −19.7 g (95% CI: −45.9, 6.5) at 12 months for PFOA. A similar pattern was observed for body mass index measurements, and no associations with length were found. After sex stratification, the inverse associations with weight and body mass index were more pronounced in boys, and no clear association was seen for girls.

***Maternal concentrations of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) and duration of breastfeeding**.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Scand J Work Environ Health. 2010;36(5):413-21.

OBJECTIVE: Perfluorooctanoate (PFOA) has been associated with impaired lactation in mice. We examined whether maternal perfluorooctanesulfonate (PFOS) and PFOA concentrations correlated with duration of breastfeeding among women. METHODS: We randomly selected 1400 pregnant women from the Danish national birth cohort (1996-2002) and measured PFOS and PFOA concentrations in early pregnancy by using high performance liquid chromatography/tandem mass spectrometry. Self-reported data on the duration of any and exclusive breastfeeding were collected twice during telephone interviews around 6 and 18 months after the birth of the child. RESULTS: The duration of breastfeeding decreased with increasing concentrations of pregnancy PFOS and PFOA among multiparous women, for whom the adjusted odds ratios (OR) for weaning before 6 months of age were 1.20 (95% CI 1.06-1.37) per 10 ng/ml increase in PFOS concentrations and 1.23 (95% CI 1.13-1.33) per 1 ng/ml increase in PFOA concentrations. No consistent association was found for primiparous women. CONCLUSIONS: These findings suggest that PFOA and PFOS may reduce the ability to lactate, but could equally reflect reverse causation since no association was seen in primiparous women.

* denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

***Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome**.

Stein C. R., Savitz D. A. and Dougan M.
Am J Epidemiol. 2009;170(7):837-46.

The authors examined the association of serum perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with self-reported pregnancy outcome in Mid-Ohio Valley residents (2000-2006) highly exposed to PFOA. Data on 1,845 pregnancies within the 5 years preceding exposure measurement were analyzed for PFOA, and data on 5,262 pregnancies were analyzed for PFOS. Generalized estimating equations were used to calculate adjusted odds ratios and 95% confidence intervals. Neither PFOA nor PFOS showed any association with miscarriage or preterm birth. Preeclampsia was weakly associated with PFOA (adjusted odds ratio = 1.3, 95% confidence interval: 0.9, 1.9) and PFOS (adjusted odds ratio = 1.3, 95% confidence interval: 1.1, 1.7) exposures above the median. PFOA was not associated with an increase in low birth weight, but PFOS showed an increased risk above the median (adjusted odds ratio = 1.5, 95% confidence interval: 1.1, 1.9) and a dose-response gradient. Birth defects were weakly associated with PFOA exposures above the 90th percentile (adjusted odds ratio = 1.7, 95% confidence interval: 0.8, 3.6). This study identified modest associations of PFOA with preeclampsia and birth defects and of PFOS with preeclampsia and low birth weight, but associations were small, limited in precision, and based solely on self-reported health outcomes.

***Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth**.
Washino N., Saijo Y., Sasaki S., Kato S., Ban S., Konishi K., Ito R., Nakata A., Iwasaki Y., Saito K., Nakazawa H. and Kishi R.
Environ Health Perspect. 2009;117(4):660-7.

BACKGROUND: Perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are man-made, ubiquitous, and persistent contaminants in the environment, wildlife, and humans. Although recent studies have shown that these chemicals interfere with fetal growth in humans, the results are inconsistent. OBJECTIVES: Our goal was to investigate the correlation between relatively low levels of PFOS and PFOA in maternal serum and birth weight and birth size. METHODS: We conducted a hospital-based prospective cohort study between July 2002 and October 2005 in Sapporo, Japan. A

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

total of 428 women and their infants were involved in the study. We obtained characteristics of the mothers and infants from self-administered questionnaire surveys and from medical records. We analyzed maternal serum samples for PFOS and PFOA by liquid chromatography-tandem mass spectrometry (LC/MS/MS). RESULTS: After adjusting for confounding factors, PFOS levels negatively correlated with birth weight [per log10 unit: beta = -148.8 g; 95% confidence interval (CI), -297.0 to -0.5 g]. In addition, analyses stratified by sex revealed that PFOS levels negatively correlated with birth weight only in female infants (per log10 unit: beta = -269.4 g; 95% CI, -465.7 to -73.0 g). However, we observed no correlation between PFOA levels and birth weight. CONCLUSION: Our results indicate that in utero exposure to relatively low levels of PFOS was negatively correlated with birth weight.

Maternal levels of perfluorinated chemicals and subfecundity.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Hum Reprod. 2009;24(5):1200-5.

BACKGROUND: Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are ubiquitous man-made compounds that are possible hormonal disruptors. We examined whether exposure to these compounds may decrease fecundity in humans. METHODS: Plasma levels of PFOS and PFOA were measured at weeks 4-14 of pregnancy among 1240 women from the Danish National Birth Cohort recruited from 1996 to 2002. For this pregnancy, women reported time to pregnancy (TTP) in five categories (<1, 1-2, 3-5, 6-12 and >12 months). Infertility was defined as having a TTP of >12 months or received infertility treatment to establish this pregnancy. RESULTS: Longer TTP was associated with higher maternal levels of PFOA and PFOS (P < 0.001). Compared with women in the lowest exposure quartile, the adjusted odds of infertility increased by 70-134 and 60-154% among women in the higher three quartiles of PFOS and PFOA, respectively. Fecundity odds ratios (FORs) were also estimated using Cox discrete-time models. The adjusted FORs were virtually identical for women in the three highest exposure groups of PFOS (FOR = 0.70, 0.67 and 0.74, respectively) compared with the lowest quartile. A linear-like trend was observed for PFOA (FOR = 0.72, 0.73 and 0.60 for three highest quartiles versus lowest quartile). When all quartiles were included in a likelihood ratio test, the trends were significant for PFOS and PFOA (P = 0.002 and P < 0.001, respectively). CONCLUSIONS: These findings suggest that PFOA and PFOS exposure at plasma levels seen in the general population may reduce fecundity; such exposure levels are common in developed countries.

Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth.
Apelberg B. J., Witter F. R., Herbstman J. B., Calafat A. M., Halden R. U., Needham L. L. and Goldman L. R.
Environ Health Perspect. 2007;115(11):1670-6.

BACKGROUND: Recent studies have reported developmental toxicity among rodents dosed with perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). OBJECTIVES: We examined the relationship between concentrations of PFOS and PFOA in cord serum (surrogates for in utero exposures) and gestational age, birth weight, and birth size in humans. METHODS: We conducted a hospital-based cross-sectional epidemiologic study of singleton deliveries in Baltimore, Maryland. Cord serum samples (n = 293) were analyzed for PFOS and PFOA by online solid-phase extraction, coupled with reversed-phase high-performance liquid chromatography-isotope dilution tandem mass spectrometry. Maternal characteristics and anthropometric measures were obtained from medical charts. RESULTS: After adjusting for potential confounders, both PFOS and PFOA were negatively associated with birth weight [per ln-unit: beta = -69 g, 95% confidence interval (CI), -149 to 10 for PFOS; beta = -104 g, 95% CI, -213 to 5 for PFOA], ponderal index (per ln-unit: beta = -0.074 g/cm(3) x 100, 95% CI, -0.123 to -0.025 for PFOS; beta = -0.070 g/cm(3) x 100, 95% CI, -0.138 to -0.001 for PFOA), and head circumference (per ln-unit: beta = -0.32 cm, 95% CI, -0.56 to -0.07 for PFOS; beta = -0.41 cm, 95% CI, -0.76 to -0.07 for PFOA). No associations were observed between either PFOS or PFOA concentrations and newborn length or gestational age. All associations were independent of cord serum lipid concentrations. CONCLUSIONS: Despite relatively low cord serum concentrations, we observed small negative associations between both PFOS and PFOA concentrations and birth weight and size. Future studies should attempt to replicate these findings in other populations.

ii. Studies that were not statistically significant

Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants - a Dutch prospective cohort study.
de Cock M., de Boer M. R., Lamoree M., Legler J. and van de Bor M.
Environ Health. 2014;13:106.

BACKGROUND: Endocrine disrupting chemicals (EDCs) present in the environment may disrupt thyroid hormones, which in early life are essential for brain development. Observational studies regarding this topic are still limited, however as the presence of

chemicals in the environment is ubiquitous, further research is warranted. The objective of the current study was to assess the association between exposure markers of various EDCs and thyroxine (T4) levels in newborns in a mother-child cohort in the Netherlands. METHODS: Exposure to dichlorodiphenyldichloroethylene (DDE), three di-2-ethylhexyl phthalate (DEHP) metabolites, hexachlorobenzene (HCB), polychlorinated biphenyl (PCB)-153, perfluorooctanesulfonic acid (PFOS), and perfluorooctanoic acid (PFOA) was determined in cord plasma or breast milk, and information on T4 levels in heel prick blood spots was obtained through the neonatal screening programme in the Netherlands. Linear regression models were composed to determine associations between each of the compounds and T4, which were stratified for gender and adjusted for a priori defined covariates. RESULTS: Mean T4 level was 86.9 nmol/L (n = 83). Girls in the highest quartile of DDE and PFOA exposure showed an increased T4 level compared to the lowest quartile with both crude and fully adjusted models (DDE > 107.50 ng/L, +24.8 nmol/L, 95% CI 0.79, 48.75; PFOA > 1200 ng/L, +38.6 nmol/L, 95% CI 13.34, 63.83). In boys a lower T4 level was seen in the second quartile of exposure for both PFOS and PFOA, however after fully adjusting the models these associations were attenuated. No effects were observed for the other compounds. CONCLUSION: DDE and perfluorinated alkyl acids may be associated with T4 in a sex-specific manner. These results should however be interpreted with caution, due to the relatively small study population. More research is warranted, as studies on the role of environmental contaminants in this area are still limited.

Menstrual cycle characteristics in fertile women from Greenland, Poland and Ukraine exposed to perfluorinated chemicals: a cross-sectional study.
Lyngso J., Ramlau-Hansen C. H., Hoyer B. B., Stovring H., Bonde J. P., Jonsson B. A., Lindh C. H., Pedersen H. S., Ludwicki J. K., Zviezdai V. and Toft G.
Hum Reprod. 2014;29(2):359-67.

STUDY QUESTION: Does perfluorooctane sulfonate (PFOS) and perfluorooctanate (PFOA) exposure disrupt the menstrual cyclicity? SUMMARY ANSWER: The female reproductive system may be sensitive to PFOA exposure, with longer menstrual cycle length at higher exposure. WHAT IS KNOWN ALREADY: PFOS and PFOA are persistent man-made chemicals. Experimental animal studies suggest they are reproductive toxicants but epidemiological findings are inconsistent. STUDY DESIGN, SIZE, DURATION: A cross-sectional study including 1623 pregnant women from the INUENDO cohort enrolled during antenatal care visits between June 2002 and May 2004 in Greenland, Poland and Ukraine. PARTICIPANTS/MATERIALS, SETTING, METHODS: Information on menstrual cycle characteristics was obtained by questionnaires together with a blood sample from each pregnant woman. Serum

concentrations of PFOS and PFOA were measured by liquid chromatography tandem mass spectrometry. Multiple imputations were performed to account for missing data. The association between PFOS/PFOA and menstrual cycle length (short cycle: </=24 days, long cycle: >/=32 days) and irregularities (>/=7 days in difference between cycles) was analyzed using logistic regression with tertiles of exposure. Estimates are given as adjusted odds ratios (ORs) with 95% confidence intervals (CIs). MAIN RESULTS AND THE ROLE OF CHANCE: Higher exposure levels of PFOA were associated with longer menstrual cycles in pooled estimates of all three countries. Compared with women in the lowest exposure tertile, the adjusted OR of long cycles was 1.8 (95% CI: 1.0; 3.3) among women in the highest tertile of PFOA exposure. No significant associations were observed between PFOS exposure and menstrual cycle characteristics. However, we observed a tendency toward more irregular cycles with higher exposure to PFOS [OR 1.7 (95% CI: 0.8; 3.5)]. The overall response rate was 45.3% with considerable variation between countries (91.3% in Greenland, 69.1% in Poland and 26.3% in Ukraine). LIMITATIONS, REASONS FOR CAUTION: Possible limitations in our study include varying participation rates across countries; a selected study group overrepresenting the most fertile part of the population; retrospective information on menstrual cycle characteristics; the determination of cut-points for all three outcome variables; and lacking information on some determinants of menstrual cycle characteristics, such as stress, physical activity, chronic diseases and gynecological disorders, thus confounding cannot be excluded. WIDER IMPLICATIONS OF THE FINDINGS: The generalizability of the study results is restricted to fertile women who manage to conceive and women who do not use oral contraceptives when getting pregnant or within 2 months before getting pregnant. To our knowledge only one previous epidemiological study has addressed the possible association between perfluorinated chemical exposure and menstrual disturbances. Though pointing toward different disturbances in cyclicity, both studies suggest that exposure to PFOA may affect the female reproductive function. This study contributes to the limited knowledge on effects of exposure to PFOA and PFOS on female reproductive function and suggests that the female reproductive system may be affected by environmental exposure to PFOA. STUDY FUNDING/COMPETING INTEREST(S): Supported by a scholarship from Aarhus University Research Foundation. The collection of questionnaire data and blood samples was part of the INUENDO project supported by The European Commission (Contract no. QLK4-CT-2001-00 202), www.inuendo.dk. The Ukrainian part of the study was possible by a grant from INTAS (project 012 2205). Determination of PFOA and PFOS in serum was part of the CLEAR study (www.inuendo.dk/clear) supported by the European Commission's 7th Framework Program (FP7-ENV-2008-1-226217). No conflict of interest declared.

Persistent environmental pollutants and couple fecundity: the LIFE study.
Buck Louis G. M., Sundaram R., Schisterman E. F., Sweeney A. M., Lynch C. D., Gore-Langton R. E., Maisog J., Kim S., Chen Z. and Barr D. B.
Environ Health Perspect. 2013;121(2):231-6.

BACKGROUND: Evidence suggesting that persistent environmental pollutants may be reproductive toxicants underscores the need for prospective studies of couples for whom exposures are measured. OBJECTIVES: We examined the relationship between selected persistent pollutants and couple fecundity as measured by time to pregnancy. METHODS: A cohort of 501 couples who discontinued contraception to become pregnant was prospectively followed for 12 months of trying to conceive or until a human chorionic gonadotrophin (hCG) test confirmed pregnancy. Couples completed daily journals on lifestyle and provided biospecimens for the quantification of 9 organochlorine pesticides, 1 polybrominated biphenyl, 10 polybrominated diphenyl ethers, 36 polychlorinated biphenyls (PCBs), and 7 perfluorochemicals (PFCs) in serum. Using Cox models for discrete time, we estimated fecundability odds ratios (FORs) and 95% CIs separately for each partner's concentrations adjusting for age, body mass index, serum cotinine, serum lipids (except for PFCs), and study site (Michigan or Texas); sensitivity models were further adjusted for left truncation or time off of contraception (≤ 2 months) before enrollment. RESULTS: The adjusted reduction in fecundability associated with standard deviation increases in log-transformed serum concentrations ranged between 18% and 21% for PCB congeners 118, 167, 209, and perfluorooctane sulfonamide in females; and between 17% and 29% for p,p -DDE and PCB congeners 138, 156, 157, 167, 170, 172, and 209 in males. The strongest associations were observed for PCB 167 (FOR 0.79; 95% CI: 0.64, 0.97) in females and PCB 138 (FOR = 0.71; 95% CI: 0.52, 0.98) in males. CONCLUSIONS: In this couple-based prospective cohort study with preconception enrollment and quantification of exposures in both female and male partners, we observed that a subset of persistent environmental chemicals were associated with reduced fecundity.

Serum vaccine antibody concentrations in children exposed to perfluorinated compounds.
Grandjean P., Andersen E. W., Budtz-Jorgensen E., Nielsen F., Molbak K., Weihe P. and Heilmann C.
JAMA. 2012;307(4):391-7.

CONTEXT: Perfluorinated compounds (PFCs) have emerged as important food contaminants. They cause immune suppression in a rodent model at serum concentrations similar to those occurring in the US population, but adverse health

effects of PFC exposure are poorly understood. OBJECTIVE: To determine whether PFC exposure is associated with antibody response to childhood vaccinations. DESIGN, SETTING, AND PARTICIPANTS: Prospective study of a birth cohort from the National Hospital in the Faroe Islands. A total of 656 consecutive singleton births were recruited during 1997-2000, [corrected] and 587 participated in follow-up through 2008. MAIN OUTCOME MEASURES: Serum antibody concentrations against tetanus and diphtheria toxoids at ages 5 and 7 years. RESULTS: Similar to results of prior studies in the United States, the PFCs with the highest serum concentrations were perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA). Among PFCs in maternal pregnancy serum, PFOS showed the strongest negative correlations with antibody concentrations at age 5 years, for which a 2-fold greater concentration of exposure was associated with a difference of -39% (95% CI, -55% to -17%) in the diphtheria antibody concentration. PFCs in the child's serum at age 5 years showed uniformly negative associations with antibody levels, especially at age 7 years, except that the tetanus antibody level following PFOS exposure was not statistically significant. In a structural equation model, a 2-fold greater concentration of major PFCs in child serum was associated with a difference of -49% (95% CI, -67% to -23%) in the overall antibody concentration. A 2-fold increase in PFOS and PFOA concentrations at age 5 years was associated with odds ratios between 2.38 (95% CI, 0.89 to 6.35) and 4.20 (95% CI, 1.54 to 11.44) for falling below a clinically protective level of 0.1 IU/mL for tetanus and diphtheria antibodies at age 7 years. CONCLUSION: Elevated exposures to PFCs were associated with reduced humoral immune response to routine childhood immunizations in children aged 5 and 7 years.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females.
Maisonet M., Nayha S., Lawlor D. A. and Marcus M.
Environ Int. 2015;82:49-60.

BACKGROUND: In some cross-sectional epidemiologic studies the shape of the association between serum concentrations of perfluoroalkyl acids (PFAAs) and lipids suggests departures from linearity. OBJECTIVES: We used statistical approaches allowing for non-linearity to determine associations of prenatal exposures of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) with lipid concentrations. METHODS: PFAAs were measured in serum from pregnant women

collected in 1991-1992 at enrollment in the Avon Longitudinal Study of Parents and Children and lipids in serum from their daughters at ages 7 (n=111) and 15 (n=88). The associations of PFAAs with lipids were first explored by cubic splines, followed by piecewise linear regressions by tertiles to obtain regression coefficients (beta) and their 95% confidence limits (95% CL) (in mg/dL per 1ng/mL). RESULTS: At age 7, total cholesterol was positively associated with prenatal PFOA concentrations in the lower tertile (beta=15.01; 95% CL=2.34, 27.69) but not with PFOA concentrations in the middle (beta=-3.63; 95% CL=-17.43, 10.16) and upper (beta=-1.58; 95% CL=-4.58, 1.42) tertiles. At age 15, a similar pattern was noted as well. Positive associations between LDL-C and prenatal PFOA concentration in the lower tertile were observed in daughters at ages 7 (beta=14.91; 95% CL=3.53, 28.12) and 15 (beta=13.93; 95% CL=0.60, 27.26). LDL-C was not associated with PFOA concentrations in the middle or upper tertile at any age. Neither HDL-C nor triglycerides was associated with prenatal PFOA exposure. Non-linear patterns of association of total cholesterol and LDL-C with prenatal PFOS were less consistently noted. CONCLUSION: Exposure to low levels of PFOA during prenatal development may alter lipid metabolism later in life. Given the small sample size further replication of the association in large independent cohorts is important.

Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case-control study in the Danish National Birth Cohort.
Liew Z., Ritz B., von Ehrenstein O. S., Bech B. H., Nohr E. A., Fei C., Bossi R., Henriksen T. B., Bonefeld-Jorgensen E. C. and Olsen J.
Environ Health Perspect. 2015;123(4):367-73.

BACKGROUND: Perfluoroalkyl substances (PFASs) are persistent pollutants found to be endocrine disruptive and neurotoxic in animals. Positive correlations between PFASs and neurobehavioral problems in children were reported in cross-sectional data, but findings from prospective studies are limited. OBJECTIVES: We investigated whether prenatal exposure to PFASs is associated with attention deficit/hyperactivity disorder (ADHD) or childhood autism in children. METHODS: Among 83,389 mother-child pairs enrolled in the Danish National Birth Cohort during 1996-2002, we identified 890 ADHD cases and 301 childhood autism cases from the Danish National Hospital Registry and the Danish Psychiatric Central Registry. From this cohort, we randomly selected 220 cases each of ADHD and autism, and we also randomly selected 550 controls frequency matched by child's sex. Sixteen PFASs were measured in maternal plasma collected in early or mid-pregnancy. We calculated risk ratios (RRs) using generalized linear models, taking into account sampling weights. RESULTS: Perfluorooctane

sulfonate (PFOS) and perfluorooctanoic acid (PFOA) were detected in all samples; four other PFASs were quantified in >/= 90% of the samples. We did not find consistent evidence of associations between mother's PFAS plasma levels and ADHD [per natural log nanograms per milliliter increase: PFOS RR = 0.87 (95% CI: 0.74, 1.02); PFOA RR = 0.98 (95% CI: 0.82, 1.16)] or autism [per natural log nanograms per milliliter increase: PFOS RR = 0.92 (95% CI: 0.69, 1.22); PFOA RR = 0.98 (95% CI: 0.73, 1.31)]. We found positive as well as negative associations between higher PFAS quartiles and ADHD in models that simultaneously adjusted for all PFASs, but these estimates were imprecise. CONCLUSIONS: In this study we found no consistent evidence to suggest that prenatal PFAS exposure increases the risk of ADHD or childhood autism in children.

Exposure to perfluoroalkyl substances and sperm DNA global methylation in Arctic and European populations.
Leter G., Consales C., Eleuteri P., Uccelli R., Specht I. O., Toft G., Moccia T., Budillon A., Jonsson B. A., Lindh C. H., Giwercman A., Pedersen H. S., Ludwicki J. K., Zviezdai V., Heederik D., Bonde J. P. and Spano M.
Environ Mol Mutagen. 2014;55(7):591-600.

Perfluoroalkyl substances (PFASs) are widely used in a variety of industrial processes and products, and have been detected globally in humans and wildlife. PFASs are suspected to interfere with endocrine signaling and to adversely affect human reproductive health. The aim of the present study was to investigate the associations between exposure to PFASs and sperm global methylation levels in a population of non-occupationally exposed fertile men. Measurements of PFASs in serum from 262 partners of pregnant women from Greenland, Poland and Ukraine, were also carried out by liquid chromatography tandem mass spectrometry. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) were detected in 97% of the blood samples. Two surrogate markers were used to assess DNA global methylation levels in semen samples from the same men: (a) average DNA methylation level in repetitive DNA sequences (Alu, LINE-1, Satalpha) quantified by PCR-pyrosequencing after bisulfite conversion; (b) flow cytometric immunodetection of 5-methyl-cytosines. After multivariate linear regression analysis, no major consistent associations between PFASs exposure and sperm DNA global methylation endpoints could be detected. However, since weak but statistically significant associations of different PFASs with DNA hypo- and hyper-methylation were found in some of the studied populations, effects of PFASs on sperm epigenetic processes cannot be completely excluded, and this issue warrants further investigation.

Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood.
Ode A., Kallen K., Gustafsson P., Rylander L., Jonsson B. A., Olofsson P., Ivarsson S. A., Lindh C. H. and Rignell-Hydbom A.
PLoS One. 2014;9(4):e95891.

BACKGROUND: The association between exposure to perfluorinated compounds (PFCs) and attention deficit hyperactivity disorder (ADHD) diagnosis has been sparsely investigated in humans and the findings are inconsistent. OBJECTIVES: A matched case-control study was conducted to investigate the association between fetal exposure to PFCs and ADHD diagnosis in childhood. METHODS: The study base comprised children born in Malmo, Sweden, between 1978 and 2000 that were followed up until 2005. Children with ADHD (n = 206) were identified at the Department of Child and Adolescent Psychiatry. Controls (n = 206) were selected from the study base and were matched for year of birth and maternal country of birth. PFC concentrations were measured in umbilical cord serum samples. The differences of the PFC concentrations between cases and controls were investigated using Wilcoxon's paired test. Possible threshold effects (above the upper quartile for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) and above limit of detection [LOD] for perfluorononanoic acid (PFNA)) were evaluated by conditional logistic regression. RESULTS: The median umbilical cord serum concentrations of PFOS were 6.92 ng/ml in the cases and 6.77 ng/ml in the controls. The corresponding concentrations of PFOA were 1.80 and 1.83 ng/ml. No associations between PFCs and ADHD were observed. Odds ratios adjusted for smoking status, parity, and gestational age were 0.81 (95% confidence interval [CI] 0.50 to 1.32) for PFOS, 1.07 (95% CI 0.67 to 1.7) for PFOA, and 1.1 (95% CI 0.75 to 1.7) for PFNA. CONCLUSIONS: The current study revealed no support for an association between fetal exposure to PFOS, PFOA, or PFNA and ADHD.

Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomes--a prospective study with long-term follow-up.
Strom M., Hansen S., Olsen S. F., Haug L. S., Rantakokko P., Kiviranta H. and Halldorsson T. I.
Environ Int. 2014;68:41-8.

Fetal exposure to persistent organic pollutants (POPs) has been linked to adverse neurodevelopment, but few studies have had follow-up beyond childhood. The purpose of this study was to examine the association of maternal serum concentrations of two perfluoroalkyl acids (perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS)), polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (p,p'-

DDE) and hexachlorobenzene (HCB) with offspring behavioural and affective disorders and scholastic achievement in a prebirth cohort study with 20 years of follow up. Between 1988 and 1989 pregnant women (n=965) were recruited for the prebirth Danish Fetal Origins 1988 (DaFO88) Cohort in Aarhus, Denmark. Perfluoroalkyl acids, PCBs, p,p'-DDE, and HCB were quantified in serum from week 30 of gestation (n=876 for perfluoroalkyl acids/872 for PCBs, p,p'-DDE, HCB). Offspring were followed up through national registries until 2011. We evaluated associations between maternal serum concentrations of these POPs and offspring neurodevelopmental outcomes, defined as: first admission diagnosis or prescription of medication until age >20 for (1) ADHD; (2) depression; and (3) scholastic achievement defined as mean grade on a standardized written examination given in the 9th grade (final exams of compulsory school in Denmark). Maternal concentrations of organochlorine substances and perfluoroalkyl acids were higher than present day levels. During the follow-up period there were 27 (3.1%) cases of ADHD and 104 (11.9%) cases of depression; the mean scholastic achievement was 6.7 (SD 2.3). Overall we found no association for maternal levels of any of the measured pollutants with offspring behavioural and affective disorders or with scholastic achievement. Our analyses based on biomarkers from a cohort of over 800 pregnant women with long-term close to complete follow-up through national registries showed little evidence of a programming effect of PFOA, PFOS, PCBs, p,p'-DDE, and HCB in relation to clinically and functionally relevant offspring neurodevelopmental outcomes.

No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland.
Vesterholm Jensen D., Christensen J., Virtanen H. E., Skakkebaek N. E., Main K. M., Toppari J., Veje C. W., Andersson A. M., Nielsen F., Grandjean P. and Jensen T. K. Reproduction. 2014;147(4):411-7.

Geographical differences in the occurrence of diseases in male reproductive organs, including malformation in reproductive tract, between Denmark and Finland have been reported. The reason for these differences is unknown, but differences in exposure to chemicals with endocrine-disrupting abilities have been suggested. Among these chemicals are perfluoro-alkylated substances (PFASs), a group of water- and grease-repellent chemicals used in outdoor clothes, cookware, food packaging, and textiles. In this study, we, therefore, investigated differences in PFAS exposure levels between Denmark and Finland and the association between cord blood PFAS levels and congenital cryptorchidism. Boys from a joint ongoing prospective birth cohort study were included. We analyzed PFAS levels in cord blood serum samples collected from 29

Danish boys with congenital cryptorchidism, 30 healthy Danish matched controls recruited from 1997 to 2001, 30 Finnish cases, and 78 Finnish healthy matched controls recruited from 1997 to 1999. Additionally, 48 Finnish cases recruited from 2000 to 2002 were included. Perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) were detected in all the 215 Danish and Finnish cord blood samples with significantly higher levels being observed in the Danish samples (medians: PFOA, 2.6 ng/ml and PFOS, 9.1 ng/ml) than in the Finnish samples (medians: PFOA, 2.1 ng/ml and PFOS, 5.2 ng/ml). We found no associations between cord blood PFOA and PFOS levels and congenital cryptorchidism after adjustment for confounders. Our data indicate that women in Denmark and Finland are generally exposed to PFOA and PFOS but there are differences in exposure levels between countries. We found no statistically significant association between cord blood PFOA and PFOS levels and congenital cryptorchidism; however, our study was small and larger studies are warranted.

First year growth in relation to prenatal exposure to endocrine disruptors - a Dutch prospective cohort study.
de Cock M., de Boer M. R., Lamoree M., Legler J. and van de Bor M.
Int J Environ Res Public Health. 2014;11(7):7001-21.

Growth in the first year of life may already be predictive of obesity later in childhood. The objective was to assess the association between prenatal exposure to various endocrine disrupting chemicals (EDCs) and child growth during the first year. Dichloro-diphenyldichloroethylene (DDE), mono(2-ethyl-5-carboxypentyl)phthalate (MECPP), mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP), mono(2-ethyl-5-oxohexyl)phthalate (MEOHP), polychlorinated biphenyl-153, perfluorooctanesulfonic acid, and perfluoro-octanoic acid were measured in cord plasma or breast milk. Data on weight, length, and head circumference (HC) until 11 months after birth was obtained from 89 mother-child pairs. Mixed models were composed for each health outcome and exposure in quartiles. For MEOHP, boys in quartile 1 had a higher BMI than higher exposed boys ($p = 0.029$). High DDE exposure was associated with low BMI over time in boys (0.8 kg/m2 difference at 11 m). Boys with high MECPP exposure had a greater HC (1.0 cm difference at 11 m) than other boys ($p = 0.047$), as did girls in the second quartile of MEHHP ($p = 0.018$) and DDE ($p < 0.001$) exposure. In conclusion, exposure to phthalates and DDE was associated with BMI as well as with HC during the first year after birth. These results should be interpreted with caution though, due to the limited sample size.

Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age.

Andersen C. S., Fei C., Gamborg M., Nohr E. A., Sorensen T. I. and Olsen J.
Am J Epidemiol. 2013;178(6):921-7.

Fetal exposure to the perfluoroalkyl acids, perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA), has been associated with lower birth weight and lower weight and body mass index (weight (kg)/height (m)(2)) in early infancy. It is, however, unclear if exposure to prenatal PFOS and PFOA has a lasting influence on growth. We estimated the associations between the maternal plasma level of PFOS or PFOA and the children's body mass index, waist circumference, and risk of overweight at 7 years of age. A total of 1,400 women were randomly selected from the Danish National Birth Cohort among those who provided blood samples early in pregnancy and gave birth to liveborn singletons in 1996-2002. Weight and height information at 7 years was available for 811 children. Multiple linear and logistic regression models were used for analyses. Maternal PFOS and PFOA concentrations were overall inversely but nonsignificantly associated with the children's body mass index, waist circumference, and risk of overweight at 7 years of age. In conclusion, plasma levels of PFOS and PFOA in pregnant women did not seem to have any appreciable influence on their children's anthropometry at this point in childhood.

Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men.
Vested A., Ramlau-Hansen C. H., Olsen S. F., Bonde J. P., Kristensen S. L., Halldorsson T. I., Becher G., Haug L. S., Ernst E. H. and Toft G.
Environ Health Perspect. 2013;121(4):453-8.

BACKGROUND: Perfluorinated alkyl acids (PFAAs), persistent chemicals with unique water-, dirt-, and oil-repellent properties, are suspected of having endocrine-disrupting activity. The PFAA compounds perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are found globally in humans; because they readily cross the placental barrier, in utero exposure may be a cause for concern. OBJECTIVES: We investigated whether in utero exposure to PFOA and PFOS affects semen quality, testicular volume, and reproductive hormone levels. METHODS: We recruited 169 male offspring (19-21 years of age) from a pregnancy cohort established in Aarhus, Denmark, in 1988-1989, corresponding to 37.6% of the eligible sons. Each man provided a semen sample and a blood sample. Semen samples were analyzed for sperm concentration, total sperm count, motility, and morphology, and blood samples were used to measure

reproductive hormones. As a proxy for in utero exposure, PFOA and PFOS were measured in maternal blood samples from pregnancy week 30. RESULTS: Multivariable linear regression analysis suggested that in utero exposure to PFOA was associated with lower adjusted sperm concentration (ptrend = 0.01) and total sperm count (ptrend = 0.001) and with higher adjusted levels of luteinizing hormone (ptrend = 0.03) and follicle-stimulating hormone (ptrend = 0.01). PFOS did not appear to be associated with any of the outcomes assessed, before or after adjustment. CONCLUSIONS: The results suggest that in utero exposure to PFOA may affect adult human male semen quality and reproductive hormone levels.

The influence of endocrine disruptors in a selected population of infertile women.
Caserta D., Bordi G., Ciardo F., Marci R., La Rocca C., Tait S., Bergamasco B., Stecca L., Mantovani A., Guerranti C., Fanello E. L., Perra G., Borghini F., Focardi S. E. and Moscarini M.
Gynecol Endocrinol. 2013;29(5):444-7.

Several studies report that endocrine disrupting chemicals (EDC) able to interfere with endocrine homeostasis may affect women's reproductive health. We analyzed EDC serum levels and nuclear receptors (NRs) expression in order to have an indication of the internal dose of biologically active compounds and a measurement of indicators of their effects, as a result of the repeated uptake from environmental source. The percentage of patients with detectable bisphenol A (BPA) concentrations was significantly higher in the infertile patients compared with fertile subjects. No significant difference was found between the groups with regard to perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), mono-ethylhexyl phthalate (MEHP) and di-(2-ethylhexyl) phthalate (DEHP) concentrations. Among infertile women, the mean expression of estrogen receptor alpha (ERalpha) and beta (Erbeta), androgen receptor (AR) and pregnane X receptor (PXR) was significantly higher than fertile patients. The mean expression of aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor gamma (PPARgamma) did not show significant differences between two groups. Patients with endometriosis had higher levels of PPARgamma than all women with other causes of infertility. This study led further support to EDC exposure as a risk factor for women's fertility.

Umbilical cord blood levels of perfluoroalkyl acids and polybrominated flame retardants.

Arbuckle T. E., Kubwabo C., Walker M., Davis K., Lalonde K., Kosarac I., Wen S. W. and Arnold D. L.
Int J Hyg Environ Health. 2013;216(2):184-94.

Perfluoroalkyl acids (PFAAs) and polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants representing two classes of environmental contaminants of toxicological concern, especially for infants. Canadian biomonitoring data on these chemicals are limited. The objectives of this study were to measure PFAAs and PBDEs in umbilical cord blood from approximately 100 hospital deliveries in Ottawa (Ontario, Canada) and examine associations with characteristics of the mother and infant. Geometric means were 1.469 ng/mL for perfluorooctanoate (PFOA) (95% confidence interval of 1.292-1.671 ng/mL), 4.443 ng/mL for perfluorooctane sulfonate (PFOS) (95% CI of 3.735-5.285 ng/mL), 0.359 ng/mL for perfluorononanoic acid (PFNA) (95% CI of 0.318-0.404 ng/mL), and 0.579 ng/mL for perfluorohexanesulfonate (PFHxS) (95% CI of 0.473-0.709 ng/mL). The final multiple regression models indicated that lower gravida, term gestational age, smoking during pregnancy and vaginal delivery were significantly associated with higher levels of PFOS. Similarly, a vaginal delivery was significantly associated with higher PFOA, while weak associations were found with lower gravida and birth weight less than 2500 g. Furthermore, higher PFNA concentrations were significantly associated with older mothers, and vaginal delivery, while weakly associated with term gestational age. Elevated PFHxS concentrations were significantly associated with smoking during pregnancy and lower gravida. Similar to reports from other countries, the preponderant PBDE congener measured in the cord blood was PBDE-47. Questions remain on why various studies have reported conflicting results on the association between PFAAs and birth weight.

Association between thyroid profile and perfluoroalkyl acids: data from NHNAES 2007-2008.
Jain R. B.
Environ Res. 2013;126:51-9.

The effect of six perfluoroalkyl acids (PFAAs), namely, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDE), perfluorohexane sulfonic acid (PFHxS), 2-(N-methyl-perfluorooctane sulfonamide) acetic acid (MPAH), and perfluorononanoic acid (PFNA) on the levels of six thyroid function variables, namely, thyroid stimulating hormone (TSH), free and total thyroxine

(FT4, TT4), free and total triiodothyronine (FT3, TT3), and thyroglobulin (TGN) was evaluated. Data from National Health and Nutrition Examination Survey for the years 2007-2008 were used for this evaluation. TSH levels increased with increase in levels of PFOA ($p<0.01$). There were no statistically significant associations between the levels of FT3, and FT4 with the levels of any of the six PFAAs. Levels of TT3 were found to increase with the levels of PFOA ($p=0.01$) and TT4 levels were found to increase with increase in PFHxS levels ($p<0.01$). Males had statistically significantly higher levels of FT3 than females and females had statistically significantly higher levels of TT4 than males. As compared to non-Hispanics whites and Hispanics, non-Hispanic blacks had lower levels of TSH, FT3, TT3, and TT4 but Hispanics had the lowest levels of TGN. Age was negatively associated with FT3 and TT3 but positively associated with FT4 and TT4. Non-smokers had higher levels of TSH and TT4 than smokers and smokers had higher levels of FT3 and TGN than non-smokers. Iodine deficiency was associated with increased levels of TSH, TT3, TT4, and TGN.

The associations between serum perfluorinated chemicals and thyroid function in adolescents and young adults.
Lin C. Y., Wen L. L., Lin L. Y., Wen T. W., Lien G. W., Hsu S. H., Chien K. L., Liao C. C., Sung F. C., Chen P. C. and Su T. C.
J Hazard Mater. 2013;244-245:637-44.

Perfluorinated chemicals (PFCs) have been widely used in a variety of products worldwide for years. However, the effect of PFCs on thyroid function has not yet been clearly defined. We recruited 567 subjects (aged 12-30 years) in a population-based cohort of adolescents and young adults with abnormal urinalysis in the childhood to determine the relationship between serum level of PFCs and the levels of serum free thyroxine (T4) and thyroid stimulating hormone (TSH). The geometric means and geometric standard deviation concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) were 2.67 (2.96) ng/ml, 7.78 (2.42) ng/ml, 1.01 (3.48) ng/ml and 5.81 (2.92) ng/ml, respectively. Differences in the levels of free T4 and TSH across different categories of PFOA, PFOS and PFUA were insignificant. After controlling for confounding factors, multiple linear regression analyses revealed mean serum level of free T4 increased significantly across categories (<60th, 60-89 and >90th percentiles) of PFNA (P for trend =0.012 in the full model). The association between PFNA and free T4 was more significant in male subjects in age group 20-30, active smokers and in those with higher body mass index in stratified analysis. Serum concentrations of PFNA were associated with serum free T4 levels in adolescents and young adults.

Sperm DNA integrity in relation to exposure to environmental perfluoroalkyl substances - a study of spouses of pregnant women in three geographical regions.
Specht I. O., Hougaard K. S., Spano M., Bizzaro D., Manicardi G. C., Lindh C. H., Toft G., Jonsson B. A., Giwercman A. and Bonde J. P.
Reprod Toxicol. 2012;33(4):577-83.

Perfluoroalkyl substances (PFASs) can interfere with male reproductive function, but evidence in humans is limited. Six hundred four fertile men (199 from Greenland, 197 from Poland and 208 from Ukraine) were enrolled in the study. We measured four PFASs in serum (PFOS, PFOA, PFNA and PFHxS) and concurrent DNA damage in spermatozoa by sperm chromatin structure assay (SCSA) and in situ terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, apoptotic markers in semen (Fas-receptor and Bcl-xL), and reproductive hormones in serum. No association between PFASs and SCSA, apoptotic markers or reproductive hormones emerged. We observed a slight increase in SHBG and TUNEL-positivity with increased PFOA exposure in men from Greenland. Thus, consistent evidence that PFAS exposure interferes with sperm DNA fragmentation, apoptosis or reproductive hormones was not found.

Association between perfluorinated compounds and time to pregnancy in a prospective cohort of Danish couples attempting to conceive.
Vestergaard S., Nielsen F., Andersson A. M., Hjollund N. H., Grandjean P., Andersen H. R. and Jensen T. K.
Hum Reprod. 2012;27(3):873-80.

BACKGROUND: Perfluorinated chemicals (PFCs) have been widely used and have emerged as important food contaminants. A recent study on pregnant women suggested that PFC exposure was associated with a longer time to pregnancy (TTP). We examined the association between serum concentrations of PFCs in females and TTP in 222 Danish first-time pregnancy planners during the years 1992-1995. METHODS: The couples were enrolled in the study when discontinuing birth control and followed for six menstrual cycles or until a clinically recognized pregnancy occurred. Fecundability ratio (FR) was calculated using discrete-time survival models. In addition, odds ratio (OR) for TTP >6 cycles was calculated. RESULTS: OR for TTP >6 cycles for those with PFC concentrations above the median were 0.96 [95% confidence interval (CI): 0.54-1.64] for perfluorooctane sulfonic acid (PFOS), the major PFC, compared with those below the median. FRs for those with PFOS concentrations above the median

were 1.05 (95% CI: 0.74-1.48) compared with those below the median. Other PFCs showed the same lack of association with TTP. The results were not affected by adjustment for covariates. PFOS and perfluorooctanoic acid concentrations were similar to those observed in a previous Danish study. CONCLUSIONS: These findings suggest that exposure to PFCs affects TTP only to a small extent, if at all.

Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants.
Okada E., Sasaki S., Saijo Y., Washino N., Miyashita C., Kobayashi S., Konishi K., Ito Y. M., Ito R., Nakata A., Iwasaki Y., Saito K., Nakazawa H. and Kishi R.
Environ Res. 2012;112:118-25.

BACKGROUND: Recent studies have shown effects of prenatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) on infants in the general environmental levels. Laboratory animal studies have shown that exposure to PFOS and PFOA is associated with immunotoxic effects. OBJECTIVES: To investigate the relationship between maternal PFOS and PFOA levels and infant allergies and infectious diseases during the first 18 months of life. Cord blood immunoglobulin (Ig) E levels were also evaluated. METHODS: We conducted a prospective cohort study of pregnant women from 2002 to 2005 in Sapporo, Japan. Maternal PFOS and PFOA levels were measured in relation to cord blood IgE concentrations (n=231) and infant allergies and infectious diseases (n=343). Characteristics of mothers and their infants were obtained from self-administered questionnaires and medical records. Development of infant allergies and infectious diseases was determined from self-administered questionnaires at 18 months of age. Concentrations of PFOS and PFOA in maternal serum and concentrations of IgE in umbilical cord serum at birth were measured. RESULTS: Cord blood IgE levels decreased significantly with high maternal PFOA concentration among female infants. However, there were no significant associations among maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis media in the 18 month-old infants (adjusted for confounders). CONCLUSIONS: Although cord blood IgE level decreased significantly with high maternal PFOA levels among female infants, no relationship was found between maternal PFOS and PFOA levels and infant allergies and infectious diseases at age in 18 months.

Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements.
Raymer J. H., Michael L. C., Studabaker W. B., Olsen G. W., Sloan C. S., Wilcosky T. and Walmer D. K.
Reprod Toxicol. 2012;33(4):419-27.

A total of 256 men were studied to evaluate whether serum concentrations of perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) impacted semen quality or reproductive hormones. Blood and semen were collected and analyzed for perfluorochemicals and reproductive and thyroid hormones. Semen quality was assessed using standard clinical methods. Linear and logistic modeling was performed with semen profile measurements as outcomes and PFOS and PFOA in semen and plasma as explanatory variables. Adjusting for age, abstinence, and tobacco use, there was no indication that PFOA or PFOS was significantly associated with volume, sperm concentration, percent motility, swim-up motility and concentration, and directional motility (a function of motility and modal progression). Follicle-stimulating hormone was not associated with either PFOA or PFOS. Luteinizing hormone was positively correlated with plasma PFOA and PFOS, but not semen PFOS. Important methodological concerns included the lack of multiple hormonal measurements necessary to address circadian rhythms.

Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study.
Whitworth K. W., Haug L. S., Baird D. D., Becher G., Hoppin J. A., Skjaerven R., Thomsen C., Eggesbo M., Travlos G., Wilson R., Cupul-Uicab L. A., Brantsaeter A. L. and Longnecker M. P.
Am J Epidemiol. 2012;175(12):1209-16.

Perfluorooctane sulfonate and perfluorooctanoic acid are perfluorinated compounds (PFCs) widely distributed in the environment. Previous studies of PFCs and birth weight are equivocal. The authors examined this association in the Norwegian Mother and Child Cohort Study (MoBa), using data from 901 women enrolled from 2003 to 2004 and selected for a prior case-based study of PFCs and subfecundity. Maternal plasma samples were obtained around 17 weeks of gestation. Outcomes included birth weight z scores, preterm birth, small for gestational age, and large for gestational age. The adjusted birth weight z scores were slightly lower among infants born to mothers in the highest quartiles of PFCs compared with infants born to mothers in the lowest quartiles:

for perfluorooctane sulfonate, β = -0.18 (95% confidence interval: -0.41, 0.05) and, for perfluorooctanoic acid, β = -0.21 (95% confidence interval: -0.45, 0.04). No clear evidence of an association with small for gestational age or large for gestational age was observed. Perfluorooctane sulfonate and perfluorooctanoic acid were each associated with decreased adjusted odds of preterm birth, although the cell counts were small. Whether some of the associations suggested by these findings may be due to a noncausal pharmacokinetic mechanism remains unclear.

Serum concentrations of major perfluorinated compounds among the general population in Korea: dietary sources and potential impact on thyroid hormones.
Ji K., Kim S., Kho Y., Paek D., Sakong J., Ha J., Kim S. and Choi K.
Environ Int. 2012;45:78-85.

Perfluorinated compounds (PFCs) have been frequently detected in both the environment and biota, and have become a growing concern. However, information is limited on the potential sources and human health implications of such exposure. We evaluated the exposure levels of 13 major PFCs among a population (n=633, >12 years of age) in a mid-sized city of Korea, and investigated for their potential dietary sources and the impact on thyroid hormone concentrations. For this purpose, we collected blood samples from a general population in Siheung, Korea and measured for 13 PFCs, total thyroxine (T4), and thyroid stimulating hormone (TSH). In addition, a questionnaire survey on diet was conducted. Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA) were detected in relatively greater concentrations than the other 9 PFCs in the blood serum. Males tend to have greater concentrations than females for most PFCs, and the concentrations were elevated as age increased up to 50s. Body mass index (BMI) was also shown to influence the serum concentrations of several PFCs. After adjustment for age, sex, and BMI, the consumption of vegetable, potato, fish/shellfish, and popcorn was identified to be significantly related with concentrations of major PFCs in blood. Among the studied PFCs, the concentrations of perfluorotridecanoic acid (PFTrDA) were negatively correlated with total T4, and positively with TSH levels, especially among females. The result of this study will provide information useful for developing public health and safety management measures for PFCs.

Perfluorinated acids and hypothyroxinemia in pregnant women.
Chan E., Burstyn I., Cherry N., Bamforth F. and Martin J. W.
Environ Res. 2011;111(4):559-64.

Perfluorinated acids (PFAs) are prominent and widespread contaminants of human blood. In animal studies there is evidence that suggests certain PFAs can disrupt thyroid hormone homeostasis. A commonly reported condition in exposed animals is hypothyroxinemia, whereby serum free thyroxine (fT4) is decreased despite normal thyroid stimulating hormone (TSH) concentrations. We designed an individually matched case-control study to investigate whether exposure to perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) was associated with hypothyroxinemia in pregnant women from Edmonton, Alberta, Canada, in 2005-2006, who underwent a "triple screen" blood test at 15-20 weeks gestation as part of ante-natal care. Thyroid hormones, fT4 and TSH, were measured in serum from 974 women, and from these we measured PFAs in the sera of 96 hypothyroxinemic cases (normal TSH, the lowest 10th percentile of fT4) and 175 controls (normal TSH, fT4 between the 50th and 90th percentiles) matched on age and referring physician. Analyses by conditional logistic regression indicated that the concentrations of PFAs in this population were not associated with hypothyroxinemia among pregnant women. The current findings do not support a causal link between PFA exposure and maternal hypothyroxinemia in the studied population.

Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort.
Christensen K. Y., Maisonet M., Rubin C., Holmes A., Calafat A. M., Kato K., Flanders W. D., Heron J., McGeehin M. A. and Marcus M.
Environ Int. 2011;37(1):129-35.

INTRODUCTION: Polyfluoroalkyl chemicals (PFCs) are commercially synthesized chemicals used in consumer products. Exposure to certain PFCs is widespread, and some PFCs may act as endocrine disruptors. We used data from the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom to conduct a nested case-control study examining the association between age at menarche, and exposure to PFCs during pregnancy. METHODS: Cases were selected from female offspring in the ALSPAC who reported menarche before the age of 11.5 years (n = 218), and controls were a random sample of remaining girls (n = 230). Serum samples taken from the girls' mothers during pregnancy (1991-1992) were analyzed using on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry for 8 PFCs. Logistic regression was used to determine

association between maternal serum PFC concentrations, and odds of earlier age at menarche. RESULTS: PFOS and PFOA were the predominant PFCs (median serum concentrations of 19.8 ng/mL and 3.7 ng/mL). All but one PFC were detectable in most samples. Total PFC concentration varied by number of births (inverse association with birth order; p-value < 0.0001) and race of the child (higher among whites; p-value = 0.03). The serum concentrations of carboxylates were associated with increased odds of earlier age at menarche; concentrations of perfluorooctane sulfonamide, the sulfonamide esters and sulfonates were all associated with decreased odds of earlier age at menarche. However, all confidence intervals included the null value of 1.0. CONCLUSIONS: ALSPAC study participants had nearly ubiquitous exposure to most PFCs examined, but PFC exposure did not appear to be associated with altered age at menarche of their offspring.

Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years.
Fei C. and Olsen J.
Environ Health Perspect. 2011;119(4):573-8.

OBJECTIVE: Potential neurotoxic effects of perfluorinated compounds (PFCs) have been reported in highly exposed animals, but whether these chemicals are neurotoxic in humans is not known. We therefore investigated whether prenatal exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfate (PFOS), two of the most prevalent PFCs, are associated with behavioral or coordination problems in early childhood. METHODS: We used data from the Danish National Birth Cohort, which enrolled mothers in early pregnancy, and we measured maternal blood levels of PFOA and PFOS using specimens drawn around 8 weeks of gestation. When the children reached 7 years of age, mothers completed the Strengths and Difficulties Questionnaire (SDQ, n=787) and the Developmental Coordination Disorder Questionnaire (DCDQ, n=526) to assess behavioral health and motor coordination of their children. SDQ scores above the 90th percentile were a priori defined to identify behavioral problems and DCDQ scores below the 10th percentile were defined as a potential DCD. RESULTS: The median concentrations of PFOS and PFOA in maternal blood were 34.4 ng/mL [interquartile range (IQR), 26.6-44.5] and 5.4 ng/mL (IQR, 4.0-7.1), respectively, similar to distributions reported for populations without occupational exposure. We found no association between higher SDQ scores and maternal levels of PFOS or PFOA, nor did we see any statistically significant association with motor coordination disorders. CONCLUSION: The findings suggest that background levels of PFOA and PFOS are not associated with behavioral and motor coordination problems in childhood.

However, effects on other developmental end points, including cognitive, attentional, and clinical mental disorders not measured in this study, cannot be ruled out.

Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Environ Res. 2010;110(8):773-7.

OBJECTIVES: To examine whether prenatal exposure to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) is associated with the occurrence of hospitalization for infectious diseases during early childhood. METHODS: We randomly selected 1400 pregnant women and their offspring from the Danish National Birth Cohort (1996-2002) and measured PFOS and PFOA levels in maternal blood during early pregnancy. Hospitalizations for infection of the offspring were identified by the linkage to the National Hospital Discharge Register through 2008. RESULTS: Hospitalizations due to infections were not associated with prenatal exposure to PFOA and PFOS. On the contrary, the relative risks of hospitalizations ranged from 0.71 to 0.84 for the three higher quartiles of maternal PFOA levels compared with the lowest, but no dose-response pattern was found. No clear pattern was observed when results were stratified by child's age at infection, with the exception of an inverse association between maternal PFC levels and risk of hospitalization during the child's first year of life. CONCLUSIONS: These findings suggest that prenatal exposure to PFOA or PFOS is not associated with increased risk of infectious diseases leading to hospitalization in early childhood.

Exploratory assessment of perfluorinated compounds and human thyroid function.
Bloom M. S., Kannan K., Spliethoff H. M., Tao L., Aldous K. M. and Vena J. E.
Physiol Behav. 2010;99(2):240-5.

Thyroid hormones play critical roles in human neurodevelopment and adult neurocognitive function. Persistent organohalogen pollutants, such as perfluorinated compounds (PFCs), may interfere with thyroid homeostasis and thus exposures to these compounds might represent risk factors for neurologic and cognitive abnormalities. In this study, serum specimens collected from thirty-one licensed anglers in New York State were analyzed for levels of thyroid stimulating hormone (TSH), free thyroxine (FT(4)), perfluorodecanoic acid (PFDA), perfluorononanoic acid (PFNA), perfluoroheptanoic acid (PFHpA), perfluorohexanesulfonate (PFHxS), perfluorooctanoic acid (PFOA), perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA),

and perfluoroundecanoic acid (PFUnDA). PFOS and PFOA occurred in the highest concentrations with geometric means of 19.6 ng/mL (95% CI 16.3-23.5) and 1.3 ng/mL (95% CI 1.2-1.5), respectively. In a cross-sectional analysis, no statistically significant associations were detected for PFCs, or their sum, with TSH or FT(4) at alpha=0.05. However, post hoc power analyses, though limited, suggested that moderate increases in sample size, to 86 and 129 subjects, might facilitate 80% power to detect statistically significant associations for FT(4) and PFDA (beta=0.09) and PFUnDA (beta=0.08), respectively. The consumption of sportfish may have contributed to PFDA (r=0.52, P=0.003) and PFUnDA (r=0.40, P=0.025) levels. This preliminary study does not indicate associations between non-occupational PFCs exposures and thyroid function. However, the possibility for weak associations for FT(4) with PFDA and PFUnDA, PFCs measured in low concentrations, is raised. Given the ubiquity of PFCs in the environment and the importance of thyroid function to neurodevelopmental and neurocognitive endpoints, a confirmatory study is warranted.

Maternal exposure to perfluorinated acids and fetal growth.
Hamm M. P., Cherry N. M., Chan E., Martin J. W. and Burstyn I.
J Expo Sci Environ Epidemiol. 2010;20(7):589-97.

The widespread detection of perfluorinated acids (PFAs) in humans and known developmental toxicity in animals has raised concern about their potential effects on human reproductive health. Our objective was to determine whether increasing maternal exposure to PFAs is associated with adverse effects on fetal growth and length of gestation in women giving birth in Alberta, Canada. We examined the concentrations of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS) in a cohort of 252 pregnant women who gave birth to live singletons. Each of the women had undergone an early second trimester prenatal screen, and her serum was analyzed for PFA concentrations. Data on infant and maternal variables were collected from the delivery record completed at birth. Adjusted changes in birth weight per natural log (ng/ml) of PFOA (median 1.5 ng/ml), PFHxS (median 0.97 ng/ml), and PFOS (median 7.8 ng/ml) were -37.4 g (95% confidence interval (CI): -86.0 to 11.2 g), 21.9 g (-23.4 to 67.2 g), and 31.3 g (-43.3 to 105.9 g), respectively. Mean birth weight z-score, standardized for gestational age and gender, length of gestation, and risk of preterm birth did not appear to be influenced by maternal PFA exposure. When PFA concentrations were divided into tertiles, similar patterns were observed. These results suggest that maternal PFA exposure has no substantial effect on fetal weight and length of gestation at the concentrations observed in this population.

Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples.

Monroy R., Morrison K., Teo K., Atkinson S., Kubwabo C., Stewart B. and Foster W. G.
Environ Res. 2008;108(1):56-62.

Perfluoroalkyl compounds (PFCs) are end-stage metabolic products from industrial flourochemicals used in the manufacture of plastics, textiles, and electronics that are widely distributed in the environment. The objective of the present study was to quantify exposure to perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorodecanoic acid (PFDeA), perfluorohexane sulfonate (PFHxS), perfluoroheptanoic acid (PFHpA), and perfluorononanoic acid (PFNA) in serum samples collected from pregnant women and the umbilical cord at delivery. Pregnant women (n=101) presenting for second trimester ultrasound were recruited and PFC residue levels were quantified in maternal serum at 24-28 weeks of pregnancy, at delivery, and in umbilical cord blood (UCB; n=105) by liquid chromatography-mass spectrometry. Paired t-test and multiple regression analysis were performed to determine the relationship between the concentrations of each analyte at different sample collection time points. PFOA and PFOS were detectable in all serum samples analyzed including the UCB. PFOS serum levels (mean+/-S.D.) were significantly higher ($p<0.001$) in second trimester maternal serum (18.1+/-10.9 ng/mL) than maternal serum levels at delivery (16.2+/-10.4 ng/mL), which were higher than the levels found in UCB (7.3+/-5.8 ng/mL; $p<0.001$). PFHxS was quantifiable in 46/101 (45.5%) maternal and 21/105 (20%) UCB samples with a mean concentration of 4.05+/-12.3 and 5.05+/-12.9 ng/mL, respectively. There was no association between serum PFCs at any time point studied and birth weight. Taken together our data demonstrate that although there is widespread exposure to PFCs during development, these exposures do not affect birth weight.

Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort.

Fei C., McLaughlin J. K., Tarone R. E. and Olsen J.
Am J Epidemiol. 2008;168(1):66-72.

Perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) are widespread persistent organic pollutants that have been associated with reduced birth weight at doses expected in many pregnant populations. The authors randomly selected 1,400 pregnant women and their newborns from the Danish National Birth Cohort (1996-2002) to investigate whether these compounds reduce organ growth. PFOS and PFOA were

measured in maternal blood samples taken early in pregnancy. Placental weight, birth length, and head and abdominal circumferences were measured shortly after birth by trained midwives or nurses. Maternal PFOA levels in early pregnancy were associated with smaller abdominal circumference and birth length. For each ng/ml increase in PFOA, birth length decreased by 0.069 cm (95% confidence interval: 0.024, 0.113) and abdominal circumference decreased by 0.059 cm (95% confidence interval: 0.012, 0.106). An inverse association was also observed between PFOA and placental weight and head circumference, and a positive association was observed with newborn ponderal index, but none of these associations was statistically significant. Maternal PFOS levels were not associated with any of the five fetal growth indicators. These findings suggest that fetal exposure to PFOA but not PFOS during organ development may affect the growth of organs and the skeleton.

Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy.
Fei C., McLaughlin J. K., Lipworth L. and Olsen J.
Environ Health Perspect. 2008;116(10):1391-5.

BACKGROUND: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are fluorinated organic compounds present in the general population at low concentrations. Animal studies have shown that they may affect neuromuscular development at high concentrations. OBJECTIVES: We investigated the association between plasma levels of PFOS and PFOA in pregnant women and motor and mental developmental milestones of their children. METHODS: We randomly selected 1,400 pairs of pregnant women and their children from the Danish National Birth Cohort. PFOS and PFOA were measured in maternal blood samples taken in early pregnancy. Apgar score was abstracted from the National Hospital Discharge Register in Denmark. Developmental milestones were reported by mothers using highly structured questionnaires when the children were around 6 months and 18 months of age. RESULTS: Mothers who had higher levels of PFOA and PFOS gave birth to children who had similar Apgar scores and reached virtually all of the development milestones at the same time as children born to mothers with lower exposure levels. Children who were born to mothers with higher PFOS levels were slightly more likely to start sitting without support at a later age. CONCLUSION: We found no convincing associations between developmental milestones in early childhood and levels of PFOA or PFOS as measured in maternal plasma early in pregnancy.

Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort.

Fei C., McLaughlin J. K., Tarone R. E. and Olsen J.
Environ Health Perspect. 2007;115(11):1677-82.

BACKGROUND: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) are man-made, persistent organic pollutants widely spread throughout the environment and human populations. They have been found to interfere with fetal growth in some animal models, but whether a similar effect is seen in humans is uncertain. OBJECTIVES: We investigated the association between plasma levels of PFOS and PFOA in pregnant women and their infants' birth weight and length of gestation. METHODS: We randomly selected 1,400 women and their infants from the Danish National Birth Cohort among those who completed all four computer-assisted telephone interviews, provided the first blood samples between gestational weeks 4 and 14, and who gave birth to a single live-born child without congenital malformation. PFOS and PFOA were measured by high performance liquid chromatography-tandem mass spectrometer. RESULTS: PFOS and PFOA levels in maternal plasma were on average 35.3 and 5.6 ng/mL, respectively. Only PFOA levels were inversely associated with birth weight (adjusted beta = -10.63 g; 95% confidence interval, -20.79 to -0.47 g). Neither maternal PFOS nor PFOA levels were consistently associated with the risk for preterm birth or low birth weight. We observed no adverse effects for maternal PFOS or PFOA levels on small for gestational age. CONCLUSION: Our nationwide cohort data suggest an inverse association between maternal plasma PFOA levels and birth weight. Because of widespread exposure to these chemicals, our findings may be of potential public health concern.

C. Studies with unclear findings

Polyfluoroalkyl chemicals and menopause among women 20-65 years of age (NHANES).
Taylor K. W., Hoffman K., Thayer K. A. and Daniels J. L.
Environ Health Perspect. 2014;122(2):145-50.

BACKGROUND: Polyfluoroalkyl chemicals (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been associated with early menopause. However, previous cross-sectional studies have lacked adequate data to investigate possible reverse causality (i.e., higher serum concentrations due to decreased excretion after menopause). OBJECTIVES: We investigated the association between PFOS,

PFOA, perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS) and age at natural menopause among women 20-65 years of age in NHANES (National Health and Nutrition Examination Survey). METHODS: We used proportional hazard models to estimate hazard ratios (HRs) for the onset of natural menopause as a function of age and serum PFC levels, and to investigate reverse causation by estimating associations between PFC levels and the rate of hysterectomy. We also used multivariable linear regression to determine whether time since menopause predicted serum PFC levels. RESULTS: After adjusting for age at survey, race/ethnicity, education, ever smoking, and parity, women with higher levels of PFCs had earlier menopause than did women with the lowest PFC levels. We observed a monotonic association with PFHxS: The HR was 1.42 (95% CI: 1.08, 1.87) for serum concentrations in tertile 2 versus tertile 1, and 1.70 (95% CI: 1.36, 2.12) for tertile 3 versus tertile 1. We also found evidence of reverse causation: PFCs were positively associated with rate of hysterectomy, and time since natural menopause was positively associated with serum PFCs. CONCLUSIONS: Our findings suggest a positive association between PFCs and menopause; however, at least part of the association may be due to reverse causation. Regardless of underlying cause, women appear to have higher PFC concentrations after menopause.

Exposure and effective dose biomarkers for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) in infertile subjects: preliminary results of the PREVIENI project.

La Rocca C., Alessi E., Bergamasco B., Caserta D., Ciardo F., Fanello E., Focardi S., Guerranti C., Stecca L., Moscarini M., Perra G., Tait S., Zaghi C. and Mantovani A.
Int J Hyg Environ Health. 2012;215(2):206-11.

Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been used as surfactants in various industry and consumer products. PFOS/PFOA are very persistent in the environment and bioaccumulate in humans. They are potential reproductive and developmental toxicants and are considered to be emerging endocrine disrupters (EDs). The Italian project PREVIENI, funded by the Italian Environment Ministry, aims to link environment and human health through the investigation of selected endocrine disrupters (EDs) exposure and associated biomarkers related to human infertility conditions. In the early PREVIENI phase, PFOS and PFOA were determined in 53 couples affected by an infertility status, enrolled in a metropolitan area, according to established inclusion criteria and informed consensus. Nuclear receptors related to chemical compounds interactions were selected as biomarkers of effect and their gene expression modulations were analyzed in human peripheral blood mononuclear cell (PBMC). Among couples, subjects not presenting infertility factors (IF--) were separated from affected subjects (IF++). Most IF-- serum samples showed

PFOS and PFOA concentrations overlapping the limit of detection (LOD) of 0.5 ng/g wet weight (ww). A substantial percentage of IF++ serum samples showed PFOS concentrations >20-fold the LOD, i.e. from 3 to 50 ng/g ww. In male (50%, n=26) and from 3 to 144 ng/g ww in female (37%, n=30) samples. PFOA values were below the LOD levels in 90% of the total samples. Peroxisome proliferator-activated receptor-γ (PPARγ) and aryl hydrocarbon receptor (AhR) showed a low level of expression in PBMC of both IF++ and IF-- groups. Whereas α and β estrogen receptors (ERα and ERβ), androgen receptor (AR), and pregnane X receptor (PXR) were all upregulated in IF++ of both sexes with respect to IF-- group. Our preliminary results related to the metropolitan area indicate that subjects affected by infertility factors tend to have both higher PFOS levels and higher gene expression of specific nuclear receptors.

Do perfluoroalkyl compounds impair human semen quality?
Joensen U. N., Bossi R., Leffers H., Jensen A. A., Skakkebaek N. E. and Jorgensen N. Environ Health Perspect. 2009;117(6):923-7.

BACKGROUND: Perfluoroalkyl acids (PFAAs) are found globally in wildlife and humans and are suspected to act as endocrine disruptors. There are no previous reports of PFAA levels in adult men from Denmark or of a possible association between semen quality and PFAA exposure. OBJECTIVES: We investigated possible associations between PFAAs and testicular function. We hypothesized that higher PFAA levels would be associated with lower semen quality and lower testosterone levels. METHODS: We analyzed serum samples for levels of 10 different PFAAs and reproductive hormones and assessed semen quality in 105 Danish men from the general population (median age, 19 years). RESULTS: Considerable levels of perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and perfluorohexane sulfonic acid were found in all young men (medians of 24.5, 4.9, and 6.6 ng/mL, respectively). Men with high combined levels of PFOS and PFOA had a median of 6.2 million normal spermatozoa in their ejaculate in contrast to 15.5 million among men with low PFOS-PFOA ($p = 0.030$). In addition, we found nonsignificant trends with regard to lower sperm concentration, lower total sperm counts, and altered pituitary-gonadal hormones among men with high PFOS-PFOA levels. CONCLUSION: High PFAA levels were associated with fewer normal sperm. Thus, high levels of PFAAs may contribute to the otherwise unexplained low semen quality often seen in young men. However, our findings need to be corroborated in larger studies.

D. Related articles

Associations of Perfluoroalkyl Substances (PFASs) with Lower Birth Weight: An Evaluation of Potential Confounding by Glomerular Filtration Rate Using a Physiologically Based Pharmacokinetic Model (PBPK).
Verner M. A., Loccisano A. E., Morken N. H., Yoon M., Wu H., McDougall R., Maisonet M., Marcus M., Kishi R., Miyashita C., Chen M. H., Hsieh W. S., Andersen M. E., Clewell H. J., 3rd and Longnecker M. P.
Environ Health Perspect. 2015.

BACKGROUND: Prenatal exposure to perfluoroalkyl substances (PFAS) has been associated with lower birth weight in epidemiologic studies. This association could be attributable to glomerular filtration rate (GFR) which is related to PFAS concentration and birth weight. OBJECTIVES: To use a physiologically based pharmacokinetic (PBPK) model of pregnancy to assess how much of the PFAS-birth weight association observed in epidemiologic studies might be attributable to GFR. METHODS: We modified a PBPK model to reflect the association of GFR with birth weight (estimated from three studies of GFR and birth weight) and used it to simulate PFAS concentrations in maternal and cord plasma. The model was run 250,000 times, with variation in parameters, to simulate a population. Simulated data were analyzed to evaluate the association between PFAS levels and birth weight due to GFR. We compared simulated estimates to those from a meta-analysis of epidemiologic data. RESULTS: The reduction in birth weight for each 1 ng/ml increase in simulated cord plasma for perfluorooctane sulfonate (PFOS) was 2.72 g (95% CI: -3.40, -2.04), and for perfluorooctanoic acid (PFOA) was 7.13 g (95% CI: -8.46, -5.80); results based on maternal plasma at term were similar. Results were sensitive to variations in PFAS level distributions and the strength of the GFR-birth weight association. In comparison, our meta-analysis of epidemiologic studies suggested that each 1 ng/ml increase in prenatal PFOS and PFOA levels was associated with 5.00 g (95% CI: -21.66, -7.78) and 14.72 g (95% CI: -8.92, -1.09) reductions in birth weight. CONCLUSION: Results of our simulations suggest that a substantial proportion of the association between prenatal PFAS and birth weight may be attributable to confounding by GFR and that confounding by GFR may be more important in studies with sample collection later in pregnancy.

Hazard quotient profiles used as a risk assessment tool for PFOS and PFOA serum levels in three distinctive European populations.

Ludwicki J. K., Goralczyk K., Strucinski P., Wojtyniak B., Rabczenko D., Toft G., Lindh C. H., Jonsson B. A., Lenters V., Heederik D., Czaja K., Hernik A., Pedersen H. S., Zvyezday V. and Bonde J. P.
Environ Int. 2015;74:112-8.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) blood levels are commonly used as biomarkers of human environmental exposure to these compounds. Many biomonitoring studies indicate 100% detection for PFOS and PFOA thus justifying a concern of possible risk for the most exposed individuals. This study addresses the predictive value of hazard quotients (HQs) calculated on the basis of serum PFOS and PFOA in male and female populations of reproductive age in Greenland, Poland and Ukraine. Overall, 2026 results of PFOS and PFOA serum concentrations (589 males, 1437 females) were obtained from the INUENDO database. HQs were calculated from the actual biomonitoring results and literature-based animal data linking toxicological outcomes and critical PFOS/PFOA serum levels. HQs for serum PFOS were calculated based on Points of Departure (PoD) at 13mugmL(-1) (cynomolgus monkeys, 183days, changes in THS and T3) and for PFOA at 7.1mugmL(-1) serum (male rats, 90days, hepatocellular necrosis, increased liver weight). Uncertainty factors were applied to reflect interspecies differences and human variability. Serum HQs were expressed as a ratio relative to the point of departure for each PFOS and PFOA. Only in the three cases of males in Greenland were there serum PFOS levels showing HQ values exceeding 1, so indicating that such serum levels may be of concern. The mean serum concentration of PFOS was significantly higher in male than in female populations. Despite significant differences between HQ profiles for PFOS and PFOA in donors from Greenland, Poland and Ukraine, the concentrations of these perfluoroalkylated compounds do not indicate a cause for concern, except for the three aforementioned cases from Greenland. This study demonstrates that the HQ approach can help to interpret human biomonitoring data and thus serve as a valuable tool in further risk assessment priority settings and may also be used as a basis for taking decisions in risk management.

PFOS and PFOA in paired urine and blood from general adults and pregnant women: assessment of urinary elimination.
Zhang T., Sun H., Qin X., Gan Z. and Kannan K.
Environ Sci Pollut Res Int. 2015;22(7):5572-9.

Although levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in human blood are well documented, information on elimination of these chemicals is limited. In this study, PFOS and PFOA were analyzed in 81 whole blood-urine paired samples from general adults and pregnant women in Tianjin, China. PFOS and PFOA were detected in 48 and 76% of adult urine (AU) samples, with geometric mean (GM) concentrations of 0.011 and 0.008 ng/mL, respectively; whereas relatively low PFOS and PFOA concentrations were found in maternal urine (MU) samples, with GM concentrations of 0.006 and 0.003 ng/mL, respectively. For PFOA, the coefficients of Pearson's correlation between whole blood concentrations and creatinine-adjusted and creatinine-unadjusted urinary concentrations were 0.348 ($p = 0.013$) and 0.417 ($p = 0.002$), respectively. The GM urinary elimination rates of PFOS (PFOSUER) and PFOA (PFOAUER) were 16 and 25%, respectively, for adults. These results indicate that urine is an important pathway of excretion of perfluoroalkyl substances (PFASs). The partitioning ratios of PFAS concentration between urine and whole blood (PFASU/B) in pregnant women (PFOSU/B, 0.0004; PFOAU/B, 0.0011) were significantly lower ($p = 0.025$ for PFOSU/B, $p = 0.017$ for PFOAU/B) than the ratios found in non-pregnant women (PFOSU/B, 0.0013; PFOAU/B, 0.0028). Furthermore, our results suggest a clear gender difference in the urinary elimination of PFOA, with male adults (31%) having significantly higher PFOAUER than that of female adults (19%). PFOSUER was significantly inversely correlated with age ($r = -0.334$, $p = 0.015$); these findings suggest that urinary elimination of PFOS is faster in young adults than in the elderly.

Serum levels of perfluoroalkyl acids (PFAAs) with isomer analysis and their associations with medical parameters in Chinese pregnant women.
Jiang W., Zhang Y., Zhu L. and Deng J.
Environ Int. 2014;64:40-7.

Perfluoroalkyl acids (PFAAs) are a group of chemicals used for many applications and widely present in the environment and humans. In this study, serum levels of PFAAs and isomers of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) were analyzed in 141 Chinese pregnant women. Among all the samples, total PFOS (Σ PFOS, mean concentration 7.32ng/mL) was predominant, followed by Σ PFOA (mean 4.78ng/mL) and perfluorodecanoate (PFDA, mean 1.45ng/mL). On average, the proportion of linear PFOS (n-PFOS) was 66.7% of Σ PFOS, which was higher than the

general population, implying that maternal women could excrete branched PFOS isomers to the fetus by transplacental transfer. Moreover, the proportion of n-PFOS decreased significantly with the increasing concentration of Σ PFOS in the serum samples ($r=-0.342$, $p<0.001$). The mean proportion of n-PFOA in the serum samples was 99.0%, which was much higher than the technical ECF (electrochemical fluorination) products (ca. 70%). The small proportion of branched isomers of PFOA suggests that there is still a source of ECF PFOA in China. Significant correlations ($p<0.005$) were observed between the concentrations of some PFAAs with certain medical parameters in the pregnant women. For example, the levels of most perfluorinated carboxylic acids (PFCAs) were found to correlate with albumin significantly, which might be a sign of immunotoxicity of these chemicals. The adverse effects of PFAA exposure to pregnant women may increase the health risk of the fetus. Interestingly, not only the PFAA concentrations but also the percentages of PFOS and PFOA isomers were correlated with certain medical parameters. This implies that the compositions of PFOS or PFOA should be considered in human health risk assessment in the future.

Assessment of fetal exposure and maternal elimination of perfluoroalkyl substances.
Zhang T. and Qin X.
Environ Sci Process Impacts. 2014;16(8):1878-81.

In this study, we estimated the body burden (BB) of perfluoroalkyl substances (PFASs) in a fetus at the time of delivery, and elimination of PFASs in female adults during pregnancy; and explored the isomer branching pattern-related placental transfer of perfluorooctane sulfonate (PFOS). The mean BB of PFASs were 3980 ng for PFOS and 2320 ng for perfluorooctanoic acid (PFOA), therefore, the average daily exposure doses via placental transfer were estimated to be 13.7 and 8.32 ng per day for PFOS and PFOA, respectively, by dividing the BB of PFASs by gestational age. The total daily elimination of PFOS and PFOA in female adults through pregnancy was 30.1 and 11.4 ng per day, which indicates that pregnancy and child birth may reduce the PFASs levels in female adults. Further, branched PFOS was more readily transferred through the placenta than linear PFOS.

Dietary exposure to perfluoroalkyl acids of specific French adult sub-populations: high seafood consumers, high freshwater fish consumers and pregnant women.
Yamada A., Bemrah N., Veyrand B., Pollono C., Merlo M., Desvignes V., Sirot V., Marchand P., Berrebi A., Cariou R., Antignac J. P., Le Bizec B. and Leblanc J. C.
Sci Total Environ. 2014;491-492:170-5.

Perfluoroalkyl acids (PFAAs) are globally found in various media, including food and especially fishery products. In the present study, the dietary exposure to 15 perfluoroalkyl acids was assessed for 3 French adult populations, namely high seafood consumers, high freshwater fish consumers, and pregnant women. Purified food extracts were analysed by LC-MS/MS and PFBA, PFPA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFTrDA, PFTeDA, PFBS, PFHxS, PFHpS, PFOS and PFDS were monitored and quantified according to the isotope dilution principle. Under lower bound (LB) hypothesis (i.e. contamination values<LOD considered as 0), high freshwater fish consumers appear as the most exposed to PFOS (7.5 ng.kg(-1) bw.d(-1)), PFUnA (1.3 ng.kg(-1) bw.d(-1)), PFDA (0.4 ng.kg(-1) bw.d(-1)) and PFHpS (0.03 ng.kg(-1) bw.d(-1)) while high seafood consumers appear as the most exposed to PFOA (1.2 ng.kg(-1) bw.d(-1)), PFNA (0.2 ng.kg(-1) bw.d(-1)) and PFHxS (0.06 ng.kg(-1) bw.d(-1)). For all considered populations, the major exposure contributors are fish, seafood and water under LB hypothesis, while dairy products, bread and crispbread are the main contributors under upper bound (UB) hypothesis. Besides this food exposure assessment, further studies are needed to assess the more global PFAA exposure, taking into account indoor and outdoor air, dust and cutaneous contact, which could be other important contributors for this particular class of chemicals.

Polybrominated diphenyl ethers and perfluoroalkyl substances in serum of pregnant women: levels, correlations, and potential health implications.
Vorkamp K., Nielsen F., Kyhl H. B., Husby S., Nielsen L. B., Barington T., Andersson A. M. and Jensen T. K.
Arch Environ Contam Toxicol. 2014;67(1):9-20.

Polybrominated diphenyl ethers (PBDEs), a group of flame retardants, and perfluoroalkyl substances (PFASs) were analysed in serum samples of pregnant women from Denmark to provide information about their exposure and to study indications of common exposure pathways. The main BDE congener was the fully brominated BDE-209 with a median value of 7.5 ng/g lipid (46 pg/mL; 9.8 pmol/g lipid). Other BDE congeners decreased in the order BDE-47 > BDE-99 > BDE-153. The summed concentration of tri- to hepta-BDEs was 7.7 ng/g lipid, i.e. in the higher end of previously reported concentrations from Europe, including plasma samples of pregnant Danish women. Total lipid contents were relatively low, on average 5.9 g/L (9.0 mmol/L). The main PFAS compound was perfluorooctane sulfonate with a median concentration of 8.4 ng/mL. Other PFASs decreased in the order perfluorooctanoic acid > perfluorononanoic acid > perfluorodecanoic acid > perfluorohexane sulfonate and resulted in a ΣPFAS of 12 ng/mL. Within each group, compounds were highly

intercorrelated with the exception of BDE-209, which was not correlated with any of the other compounds. No correlations were found either between PFASs and PBDEs suggesting different sources of exposure and/or pharmacokinetic and metabolisation processes. PBDE and PFAS concentrations were in the range associated with adverse effects in some epidemiological studies.

Exposure to endocrine disrupters and nuclear receptor gene expression in infertile and fertile women from different Italian areas.
La Rocca C., Tait S., Guerranti C., Busani L., Ciardo F., Bergamasco B., Stecca L., Perra G., Mancini F. R., Marci R., Bordi G., Caserta D., Focardi S., Moscarini M. and Mantovani A.
Int J Environ Res Public Health. 2014;11(10):10146-64.

Within the PREVIENI project, infertile and fertile women were enrolled from metropolitan, urban and rural Italian areas. Blood/serum levels of several endocrine disrupters (EDs) (perfluorooctane sulfonate, PFOS; perfluorooctanoic acid, PFOA; di-2-ethylhexyl-phthalate, DEHP; mono-(2-ethylhexyl)-phthalate, MEHP; bisphenol A, BPA) were evaluated concurrently with nuclear receptors (NRs) gene expression levels (ERa, ERb, AR, AhR, PPARg, PXR) in peripheral blood mononuclear cells (PBMCs). Infertile women from the metropolitan area displayed significantly higher levels of: BPA compared to fertile women (14.9 vs. 0.5 ng/mL serum); BPA and MEHP compared to infertile women from urban and rural areas; enhanced expression levels of NRs, except PPARg. Infertile women from urban and rural areas had PFOA levels significantly higher than those from metropolitan areas. Our study indicates the relevance of the living environment when investigating the exposure to EDs and the modulation of the NR panel in PBMC as a suitable biomarker of the effect, to assess the EDs impact on reproductive health.

Perfluorinated chemicals: differential toxicity, inhibition of aromatase activity and alteration of cellular lipids in human placental cells.
Gorrochategui E., Perez-Albaladejo E., Casas J., Lacorte S. and Porte C.
Toxicol Appl Pharmacol. 2014;277(2):124-30.

The cytotoxicity of eight perfluorinated chemicals (PFCs), namely, perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorododecanoic acid (PFDoA), perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS) and perfluorooctanesulfonate (PFOS) was assessed in the human placental choriocarcinoma cell line JEG-3. Only the long chain PFCs--PFOS, PFDoA, PFNA,

PFOA--showed significant cytotoxicity in JEG-3 cells with EC50 values in the range of 107 to 647 muM. The observed cytotoxicity was to some extent related to a higher uptake of the longer chain PFCs by cells (PFDoA>PFOS>>PFNA>PFOA>PFHxA). Moreover, this work evidences a high potential of PFOS, PFOA and PFBS to act as aromatase inhibitors in placental cells with IC50s in the range of 57-80 muM, the inhibitory effect of PFBS being particularly important despite the rather low uptake of the compound by cells. Finally, exposure of JEG-3 cells to a mixture of the eight PFCs (0.6 muM each) led to a relative increase (up to 3.4-fold) of several lipid classes, including phosphatidylcholines (PCs), plasmalogen PC and lyso plasmalogen PC, which suggests an interference of PFCs with membrane lipids. Overall, this work highlights the ability of the PFC mixture to alter cellular lipid pattern at concentrations well below those that generate toxicity, and the potential of the short chain PFBS, often considered a safe substitute of PFOS, to significantly inhibit aromatase activity in placental cells.

Maternal serum concentrations of per- and polyfluoroalkyl substances and their predictors in years with reduced production and use.
Berg V., Nost T. H., Huber S., Rylander C., Hansen S., Veyhe A. S., Fuskevag O. M., Odland J. O. and Sandanger T. M.
Environ Int. 2014;69:58-66.

Determining maternal concentrations of per- and polyfluoroalkyl substances (PFASs) and the relative impact of various demographic and dietary predictors is important for assessing fetal exposure and for developing proper lifestyle advisories for pregnant women. This study was conducted to investigate maternal PFAS concentrations and their predictors in years when the production and use of several PFASs declined, and to assess the relative importance of significant predictors. Blood from 391 pregnant women participating in The Northern Norway Mother-and-Child Contaminant Cohort Study (MISA) was collected in the period 2007-2009 and serum analyses of 26 PFASs were conducted. Associations between PFAS concentrations, sampling date, and demographic and dietary variables were evaluated by multivariate analyses and linear models including relevant covariates. Parity was the strongest significant predictor for all the investigated PFASs, and nulliparous women had higher concentrations compared to multiparous women (10 ng/mL versus 4.5 ng/mL in median PFOS, respectively). Serum concentrations of PFOS and PFOA of women recruited day 1-100 were 25% and 26% higher, respectively, compared to those women recruited in the last 167 days of the study (day 601-867), and the concentrations of PFNA, PFDA and PFUnDA increased with age. Dietary predictors explained 0-17% of the variation in concentrations for the different PFASs. Significantly elevated concentrations of PFOS, PFNA, PFDA and PFUnDA were found among high consumers of marine food. The concentrations of

PFHxS, PFHpS and PFNA were also increased in high consumers of game and elevated concentrations of PFHpS and PFOS were detected in high consumers of white meat. Study subjects with a high intake of salty snacks and beef had significantly higher concentrations of PFOA. The present study demonstrates that parity, sampling date and birth year are the most important predictors for maternal PFAS concentrations in years following a decrease in production and use of several PFASs. Further, dietary predictors of PFAS concentrations were identified and varied in importance according to compound.

Population variation in biomonitoring data for persistent organic pollutants (POPs): an examination of multiple population-based datasets for application to Australian pooled biomonitoring data.
Aylward L. L., Green E., Porta M., Toms L. M., Den Hond E., Schulz C., Gasull M., Pumarega J., Conrad A., Kolossa-Gehring M., Schoeters G. and Mueller J. F.
Environ Int. 2014;68:127-38.

BACKGROUND: Australian national biomonitoring for persistent organic pollutants (POPs) relies upon age-specific pooled serum samples to characterize central tendencies of concentrations but does not provide estimates of upper bound concentrations. This analysis compares population variation from biomonitoring datasets from the US, Canada, Germany, Spain, and Belgium to identify and test patterns potentially useful for estimating population upper bound reference values for the Australian population. METHODS: Arithmetic means and the ratio of the 95th percentile to the arithmetic mean (P95:mean) were assessed by survey for defined age subgroups for three polychlorinated biphenyls (PCBs 138, 153, and 180), hexachlorobenzene (HCB), p,p-dichlorodiphenyldichloroethylene (DDE), 2,2',4,4' tetrabrominated diphenylether (PBDE 47), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). RESULTS: Arithmetic mean concentrations of each analyte varied widely across surveys and age groups. However, P95:mean ratios differed to a limited extent, with no systematic variation across ages. The average P95:mean ratios were 2.2 for the three PCBs and HCB; 3.0 for DDE; 2.0 and 2.3 for PFOA and PFOS, respectively. The P95:mean ratio for PBDE 47 was more variable among age groups, ranging from 2.7 to 4.8. The average P95:mean ratios accurately estimated age group-specific P95s in the Flemish Environmental Health Survey II and were used to estimate the P95s for the Australian population by age group from the pooled biomonitoring data. CONCLUSIONS: Similar population variation patterns for POPs were observed across multiple surveys, even when absolute concentrations differed widely. These patterns can be used to estimate population upper bounds when only pooled sampling data are available.

Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011.

Okada E., Kashino I., Matsuura H., Sasaki S., Miyashita C., Yamamoto J., Ikeno T., Ito Y. M., Matsumura T., Tamakoshi A. and Kishi R.
Environ Int. 2013;60:89-96.

Perfluoroalkyl acids (PFAAs) are persistent organic pollutants that are used in a wide range of consumer products. Recent epidemiological studies have shown that prenatal exposure to toxic levels of PFAAs in the environment may adversely affect fetal growth and humoral immune response in infants and children. Here we have characterized levels of prenatal exposure to PFAA between 2003 and 2011 in Hokkaido, Japan, by measuring PFAA concentrations in plasma samples from pregnant women. The study population comprised 150 women who enrolled in a prospective birth cohort study conducted in Hokkaido. Eleven PFAAs were measured in maternal plasma samples using simultaneous analysis by ultra-performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. At the end of the study, in 2011, age- and parity-adjusted mean concentrations of perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) were 1.35ng/mL, 1.26ng/mL, 0.66ng/mL, 1.29ng/mL, 0.25ng/mL, 0.33ng/mL, 0.28ng/mL, and 3.86ng/mL, respectively. Whereas PFOS and PFOA concentrations declined 8.4%/y and 3.1%/y, respectively, PFNA and PFDA levels increased 4.7%/y and 2.4%/y, respectively, between 2003 and 2011. PFUnDA, PFDoDA, and PFTrDA were detected in the vast majority of maternal samples, but no significant temporal trend was apparent. Future studies must involve a larger population of pregnant women and their children to determine the effects of prenatal exposure to PFAA on health outcomes in infants and children.

Cumulative health risk assessment of 17 perfluoroalkylated and polyfluoroalkylated substances (PFASs) in the Swedish population.
Borg D., Lund B. O., Lindquist N. G. and Hakansson H.
Environ Int. 2013;59:112-23.

Humans are simultaneously exposed to a multitude of chemicals. Human health risk assessment of chemicals is, however, normally performed on single substances, which may underestimate the total risk, thus bringing a need for reliable methods to assess the risk of combined exposure to multiple chemicals. Per- and polyfluoroalkylated

substances (PFASs) is a large group of chemicals that has emerged as global environmental contaminants. In the Swedish population, 17 PFASs have been measured, of which the vast majority lacks human health risk assessment information. The objective of this study was to for the first time perform a cumulative health risk assessment of the 17 PFASs measured in the Swedish population, individually and in combination, using the Hazard Index (HI) approach. Swedish biomonitoring data (blood/serum concentrations of PFASs) were used and two study populations identified: 1) the general population exposed indirectly via the environment and 2) occupationally exposed professional ski waxers. Hazard data used were publicly available toxicity data for hepatotoxicity and reproductive toxicity as well as other more sensitive toxic effects. The results showed that PFASs concentrations were in the low ng/ml serum range in the general population, reaching high ng/ml and low mug/ml serum concentrations in the occupationally exposed. For those congeners lacking toxicity data with regard to hepatotoxicity and reproductive toxicity read-across extrapolations was performed. Other effects at lower dose levels were observed for some well-studied congeners. The risk characterization showed no concern for hepatotoxicity or reproductive toxicity in the general population except in a subpopulation eating PFOS-contaminated fish, illustrating that high local exposure may be of concern. For the occupationally exposed there was concern for hepatotoxicity by PFOA and all congeners in combination as well as for reproductive toxicity by all congeners in combination, thus a need for reduced exposure was identified. Concern for immunotoxicity by PFOS and for disrupted mammary gland development by PFOA was identified in both study populations as well as a need of additional toxicological data for many PFAS congeners with respect to all assessed endpoints.

Neonatal-maternal factors and perfluoroalkyl substances in cord blood.
Lien G. W., Huang C. C., Wu K. Y., Chen M. H., Lin C. Y., Chen C. Y., Hsieh W. S. and Chen P. C.
Chemosphere. 2013;92(7):843-50.

Perfluoroalkyl substances (PFASs) can cross the placenta, enter fetal circulation, and were found to correlate with adverse fetal growth. However, determinants of cord blood PFASs are not fully characterized. The study aimed to explore the association between PFASs and neonatal-maternal factors within a Taiwanese birth cohort. We selected subjects from Taiwan Birth Panel Study, which enrolled 486 infant-mother pairs in 2004-2005. We collected cord blood and analyzed perfluorooctanoic acid (PFOA), perfluorooctanyl sulfonate (PFOS), perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUA) using a simple protein precipitation and an ultra-high performance liquid chromatography/tandem mass spectrometry. We retrieved

information pertaining to maternal socio-demographics, lifestyle- and dietary-related factors through structured questionnaires during the postpartum hospital stay. A total of 439 subjects, with 90% response rate, have completed serum analysis and questionnaire survey. The median concentrations for PFOA, PFOS, PFNA, and PFUA in cord blood were 1.86, 5.67, 3.00, and 13.5ngmL(-1), respectively. After adjusting for potential confounders, multiple linear regression models revealed that log10-PFOA was positively associated with maternal age (beta=0.011) and negatively associated with multiparity (beta=-0.044). Log10-PFOS was negatively correlated with birth weight (beta=-0.011) and higher maternal education (senior high school: beta=-0.067; university: beta=-0.088). Log10-PFUA tended to negatively associate with gender, male infants (beta=-0.075), and using cosmetics during pregnancy (beta=-0.065). Interestingly, presence of cockroaches in the home was positively associated with log10-PFOA (beta=0.041) and 1og10-PFNA (beta=0.123). In conclusion, this study demonstrated several factors to correlate with cord blood PFASs and further investigation are still needed for confirmation of exposure routes.

Effect of pregnancy on the levels of selected perfluoroalkyl compounds for females aged 17-39 years: data from National Health and Nutrition Examination Survey 2003-2008.
Jain R. B.
J Toxicol Environ Health A. 2013;76(7):409-21.

The presence of perfluoroalkyl chemicals (PFC) in maternal serum may pose a risk to the developing fetus. A large-scale study to evaluate the extent of exposure to PFC in pregnant and nonpregnant females in the United States has not been conducted. The impact of pregnancy on the concentration levels of perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorooctanoate (PFOA), and perfluorooctane sulfonate (PFOS) was assessed by analyzing data (n = 1079) from National Health and Nutrition Examination Survey (NHANES) for the years 2003-2008 for females aged 17-39 yr. While pregnant females possessed lower serum concentrations of all 4 PFC than nonpregnant females, only the differences for PFOS were significant (9.6 vs. 11.8 ng/ml). Those mothers who breast-fed at least one child displayed significantly lower levels of PFOA (2.6 vs. 3.1 ng/ml) than those with non-breast-fed infants. The concentration levels of PFNA and PFOA decreased with increase in number of live births. While levels of PFHxS and PFOS markedly fell over the period 2003-2008, the levels of PFNA rose over the same time period. There was nonlinear elevation in levels of PFHxS and PFOS with age. Smoking was associated with increased levels of PFNA and PFOA. There was a significant, positive association between total cholesterol and PFOS as well as for serum albumin with PFHxS and

PFOS. Elevated levels of PFNA and PFOA were associated with a rise in serum protein. Further studies are needed to adequately explain why smoking was associated with increased levels of PFNA and PFOA.

Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds.
Ode A., Rylander L., Lindh C. H., Kallen K., Jonsson B. A., Gustafsson P., Olofsson P., Ivarsson S. A. and Rignell-Hydbom A.
Environ Sci Pollut Res Int. 2013;20(11):7970-8.

In recent years, some perfluorinated compounds (PFCs) have been identified as potentially hazardous substances which are harmful to the environment and human health. According to limited data, PFC levels in humans could be influenced by several determinants. However, the findings are inconsistent. In the present study, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were measured in paired maternal and cord serum samples (N=237) collected between 1978 and 2001 in Southern Sweden to study the relationship between these and to investigate several potential determinants of maternal and fetal exposure to PFCs. Time trends of PFCs in Swedish women were also evaluated. The study is a part of the Fetal Environment and Neurodevelopment Disorders in Epidemiological Research project. PFOS, PFOA, and PFNA levels (median) were higher in maternal serum (15, 2.1, and 0.24 ng/ml, respectively) than in cord serum (6.5, 1.7, and 0.20 ng/ml, respectively). PFC levels were among the highest in women originating from the Nordic countries and the lowest in women from the Middle East, North Africa, and sub-Saharan Africa. Multiparous women had lower serum PFOA levels (1.7 ng/ml) than primiparous women (2.4 ng/ml). Maternal age, body mass index, cotinine levels, and whether women carried male or female fetuses did not affect serum PFC concentrations. Umbilical cord serum PFC concentrations showed roughly similar patterns as the maternal except for the gestational age where PFC levels increased with advancing gestational age. PFOS levels increased during the study period in native Swedish women. In summary, PFOS levels tend to increase while PFOA and PFNA levels were unchanged between 1978 and 2001 in our study population. Our results demonstrate that maternal country of origin, parity, and gestational age might be associated with PFC exposure.

Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women.

Brantsaeter A. L., Whitworth K. W., Ydersbond T. A., Haug L. S., Haugen M., Knutsen H. K., Thomsen C., Meltzer H. M., Becher G., Sabaredzovic A., Hoppin J. A., Eggesbo M. and Longnecker M. P.

Environ Int. 2013;54:74-84.

BACKGROUND: Perfluoroalkyl substances (PFASs) are widespread pollutants that have been associated with adverse health effects although not on a consistent basis. Diet has been considered the main source of exposure. The aim of the present study was to identify determinants of four plasma PFASs in pregnant Norwegian women. METHODS: This study is based in the Norwegian Mother and Child Cohort Study (MoBa) conducted by the Norwegian Institute of Public Health. Our sample included 487 women who enrolled in MoBa from 2003 to 2004. A questionnaire regarding sociodemographic, medical, and reproductive history was completed at 17 weeks of gestation and a dietary questionnaire was completed at 22 weeks of gestation. Maternal plasma samples were obtained around 17 weeks of gestation. Plasma concentrations of four PFASs (perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA)) were examined in relation to demographic, lifestyle, dietary, and pregnancy-related covariates. Predictors were identified by optimizing multiple linear regression models using Akaike's information criterion (AIC). RESULTS: Parity was the determinant with the largest influence on plasma PFAS concentrations, with r(2) between 0.09 and 0.32 in simple regression models. In optimal multivariate models, when compared to nulliparous women, parous women had 46%, 70%, 19%, and 62% lower concentrations of PFOS, PFOA, PFHxS, and PFNA respectively ($p<0.001$ except for PFHxS, $p<0.01$). In all these models, duration of breastfeeding was associated with reduced PFAS levels. PFOA showed the largest reduction from breastfeeding, with a 2-3% reduction per month of breastfeeding in typical cases. Levels of PFOS, PFOA, and PFNA increased with time since most recent pregnancy. While pregnancy-related factors were the most important predictors, diet was a significant factor explaining up to 4% of the variance. One quartile increase in estimated dietary PFAS intake was associated with plasma PFOS, PFOA, PFHxS, and PFNA concentration increases of 7.2%, 3.3%, 5.8% and 9.8%, respectively, resulting in small, although non-trivial absolute changes in PFAS concentrations. CONCLUSION: Previous pregnancies and breastfeeding duration were the most important determinants of PFASs in this sample of pregnant women.

Partition of perfluoroalkyl substances (PFASs) in whole blood and plasma, assessed in maternal and umbilical cord samples from inhabitants of arctic Russia and Uzbekistan.
Hanssen L., Dudarev A. A., Huber S., Odland J. O., Nieboer E. and Sandanger T. M.
Sci Total Environ. 2013;447:430-7.

Perfluoroalkyl substances (PFASs) are ubiquitous in the environment world-wide. Our overall objective was to assess the exposure to PFASs experienced by delivering women and their new-borns in the industrial city of Norilsk (arctic Russia) and the rural Aral Sea region of Uzbekistan, with the secondary objective of evaluating the distribution of PFASs between blood cell and plasma fractions. Six PFASs were detected in every sample from Norilsk city with the plasma concentration sequence of: PFOS>>PFOA>PFNA>FOSA>PFHxS>PFUnDA. In the Uzbekistani samples, only PFOS was reported above the MDL (0.08 ng/mL). The median plasma concentrations of PFOS of 11.0 ng/mL for the Norilsk mothers was comparable to that reported for western countries, while that for Uzbekistan was considerably lower (0.23 ng/mL). Apparent increases in the maternal-cord concentration ratios for both whole blood and plasma were evident with the length of the carbon chain for both the carboxylate and the sulfonate PFASs. The median value of this ratio for FOSA in plasma was the lowest, while that for whole blood was the highest. Other than for FOSA, the observed plasma-whole blood concentration ratios for maternal and umbilical cord blood were consistent with a priori calculations using appropriate packed cell and plasma volumes for neonates and pregnant women at term. Clearly FOSA favored whole blood, and acid-base equilibrium calculations suggested that the resonance-stabilized sulfonamidate ion resides in the blood cell fraction. Thus for PFASs and related compounds with pK values with magnitudes comparable to physiological pH, it is pertinent to measure the cell-associated fraction (separately or as whole blood). Our study illustrates that consideration of both the physico-chemical properties of the contaminants and the physiological attributes of blood matrices were helpful in the interpretation of our findings.

Circulating maternal perfluoroalkyl substances during pregnancy in the C8 Health Study.
Javins B., Hobbs G., Ducatman A. M., Pilkerton C., Tacker D. and Knox S. S.
Environ Sci Technol. 2013;47(3):1606-13.

Perfluoroalkyl substances are manmade chemicals used in many consumer products and have become ubiquitous in the environment. Animal studies and a limited number of human studies have demonstrated developmental effects in offspring exposed to

perfluoroalkyl substances in utero, but the implications of timing of in utero exposure have not been systematically investigated. The present study investigated variation in perfluorocarbon levels of 9952 women of childbearing age who had been exposed to perfluorooctanoic acid (PFOA) in drinking water contaminated by industrial waste. An analysis of variance with contrast was performed to compare the levels of PFOA and perfluorooctanesulfonic acid (PFOS) in pregnant and nonpregnant women overall and during each trimester of pregnancy. We found that pregnant women had lower circulating PFOA and PFOS concentrations in peripheral blood than nonpregnant women and that PFOA levels were consistently lower throughout all trimesters for pregnancy, suggesting transfer to the fetus at an early stage of gestation. These results are discussed in the context of the endocrine-disrupting properties of perfluoroalkyl substances that have been characterized in animal and human studies. Our conclusion is that further, systematic study of the potential implications of intrauterine perfluorocarbon exposure during critical periods of fetal development is urgently needed.

Development of PBPK models for PFOA and PFOS for human pregnancy and lactation life stages.
Loccisano A. E., Longnecker M. P., Campbell J. L., Jr., Andersen M. E. and Clewell H. J., 3rd
J Toxicol Environ Health A. 2013;76(1):25-57.

Perfluoroalkyl acid carboxylates and sulfonates (PFAA) have many consumer and industrial applications. Developmental toxicity studies in animals have raised concern about potential reproductive/developmental effects of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS); however, in humans conflicting results have been reported for associations between maternal PFAA levels and these outcomes. Risk assessments and interpretation of available human data during gestation and lactation are hindered due to lack of a framework for understanding and estimating maternal, fetal, and neonatal pharmacokinetics (PK). Physiologically based pharmacokinetic (PBPK) models were developed for PFOA and PFOS for the gestation and lactation life stages in humans to understand how the physiological changes associated with development affect pharmacokinetics of these compounds in the mother, fetus, and infant. These models were derived from PBPK models for PFOA/PFOS that were previously developed for adult humans and rats during gestation and lactation and from existing human pregnancy and lactation models developed for other chemicals. The models simulated PFOA and PFOS concentrations in fetal, infant, and maternal plasma and milk, were compared to available data in humans, and also were used to estimate maternal exposure. The models reported here identified several

research needs, which include (1) the identification of transporters involved in renal resorption to explain the multiyear half-lives of these compounds in humans, (2) factors affecting clearance of PFOA/PFOS during gestation and lactation, and (3) data to estimate clearance of PFOA/PFOS in infants. These models may help address concerns regarding possible adverse health effects due to PFOA/PFOS exposure in the fetus and infant and may be useful in comparing pharmacokinetics across life stages.

Placental transfer of persistent organic pollutants: a preliminary study on mother-newborn pairs.
Porpora M. G., Lucchini R., Abballe A., Ingelido A. M., Valentini S., Fuggetta E., Cardi V., Ticino A., Marra V., Fulgenzi A. R. and De Felip E.
Int J Environ Res Public Health. 2013;10(2):699-711.

The aim of this study was to characterize the placental transfer of some environmental pollutants, and to explore the possibility of quantitatively predicting in utero exposure to these contaminants from concentrations assessed in maternal blood. Levels of toxic substances such as pesticides (p,p'-DDE, beta-HCH, and HCB), polychlorinated biphenyls (PCBs), perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA) were determined in serum samples of 38 pregnant women living in Rome and in samples of cord blood from their respective newborns. The study was carried out in the years 2008-2009. PCB mean concentrations in maternal serum and cord serum ranged from 0.058 to 0.30, and from 0.018 to 0.064 ng/g . fw respectively. Arithmetic means of PFOS and PFOA concentrations in mothers and newborns were 3.2 and 1.4 ng/g . fw, and 2.9 and 1.6 ng/g . fw. A strong correlation was observed between concentrations in the maternal and the foetal compartment for PFOS (Spearman r = 0.74, p < 0.001), PFOA (Spearman r = 0.70, p < 0.001), PCB 153 (Spearman r = 0.60, p < 0.001), HCB (Spearman r = 0.68, p < 0.001), PCB 180 (Spearman r = 0.55, p = 0.0012), and p,p'-DDE (Spearman r = 0.53, p = 0.0099). A weak correlation (p < 0.1) was observed for PCBs 118 and 138.

Analysis of polyfluoroalkyl substances and bisphenol A in dried blood spots by liquid chromatography tandem mass spectrometry.
Ma W., Kannan K., Wu Q., Bell E. M., Druschel C. M., Caggana M. and Aldous K. M.
Anal Bioanal Chem. 2013;405(12):4127-38.

Dried blood spots (DBS), collected as part of the newborn screening program (NSP) in the USA, is a valuable resource for studies on environmental chemical exposures and associated health outcomes in newborns. Nevertheless, determination of concentrations of environmental chemicals in DBS requires assays with great sensitivity, as the typical

volume of blood available on a DBS with 16-mm diameter disc is approximately 50 muL. In this study, we developed a liquid-liquid extraction and high-performance liquid chromatography/tandem mass spectrometry method for the detection of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and bisphenol A (BPA) in DBS. The method was validated for accuracy, precision, and sensitivity, by spiking of target chemicals at different levels on Whatman 903 filter cards, which is used in the collection of DBS by the NSP. Contamination arising from collection, storage, and handling of DBS is an important issue to be considered in the analysis of trace levels of environmental chemicals in DBS. For the evaluation of the magnitude of background contamination, field blanks were prepared from unspotted portions of DBS filter cards collected by the NSP. The method was applied for the measurement of PFOS, PFOA, and BPA in 192 DBS specimens provided by NSP of New York State. PFOS and PFOA were detected in 100 % of the specimens analyzed. The concentrations of PFOS and PFOA measured in DBS were similar to those reported earlier in the whole blood samples of newborns. BPA was also found in 86 % of the specimens at concentrations ranging from 0.2 to 36 ng/mL (excluding two outliers). Further studies are needed to evaluate the sources of BPA exposures and health outcomes in newborns.

Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid.
Henry N. D. and Fair P. A.
J Appl Toxicol. 2013;33(4):265-72.

Concern with increasing levels of emerging contaminants exists on a global scale. Three commonly observed emerging environmental contaminants: triclosan (2,4,4-trichloro-2'-hydroxydiphenyl ether), a synthetic, broad-spectrum antibacterial agent, and perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), used in stain- and water-resistant treatments, have become distributed ubiquitously across ecosystems and have been detected in wildlife and humans. MCF-7 BOS human breast cancer cells were used to investigate the potential for cytotoxicity, estrogenicity and anti-estrogenicity of these three compounds at environmentally relevant concentrations using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetr azolium, inner salt assay (MTS) and the E-SCREEN bioassay. The doses used were 0.002-200 microg ml(-1) for triclosan and 0.03-30 microg ml(-1) for PFOS and PFOA. Quantitative results from the MTS assay revealed no significant cytotoxicity at lower concentrations for any of the test compounds; however, both triclosan and PFOA were cytotoxic at the highest concentrations examined (100-200 and 30 microg ml(-1), respectively), while PFOS showed no significant cytotoxicity at any of the concentrations tested. Positive estrogenic responses ($P < 0.05$) were elicited from the

E-SCREEN at all concentrations examined for triclosan and PFOA and at 30 microg ml(-1) for PFOS. Further, significant anti-estrogenic activity (P < 0.05) was detected for all compounds tested at all concentrations when cells were co-exposed with 10(-9) m 17-beta estradiol (E(2)). The overall results demonstrated that triclosan, PFOS and PFOA have estrogenic activities and that co-exposure to contaminants and E(2) produced anti-estrogenic effects. Each of these compounds could provide a source of xenoestrogens to humans and wildlife in the environment. Published 2011. This article is a US Government work and is in the public domain in the USA.

Perfluoroalkyl substances in human milk: a first survey in Italy.
Barbarossa A., Masetti R., Gazzotti T., Zama D., Astolfi A., Veyrand B., Pession A. and Pagliuca G.
Environ Int. 2013;51:27-30.

Due to their widespread diffusion, perfluoroalkyl substances (PFASs) have been frequently found in the environment and in several animal species. It has been demonstrated that they can easily reach also humans, mainly through diet. Being lactation a major route of elimination of these contaminants, their occurrence in human milk is of particular interest, especially considering that it generally represents the unique food source for newborns. Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the two most important compounds of this family, have been frequently found in human milk at variable concentrations, but still limited data are available. The present study, the first conducted in Italy capable to detect these pollutants at ultra-trace levels by UPLC-MS/MS, confirmed the role of lactation as a relevant source of exposure for breastfed children. The measured concentrations ranged between 15 and 288 ng/L for PFOS and between 24 and 241 ng/L for PFOA. Moreover, mean concentrations and frequencies of both analytes resulted higher in milk samples provided by primiparous women, suggesting that the risk of intake might be higher for first-borns. Finally, comparing these results with previous data, PFOS gradual decrease over time since year 2000 was confirmed.

Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study.
Stein C. R., Wolff M. S., Calafat A. M., Kato K. and Engel S. M.
Reprod Toxicol. 2012;34(3):312-6.

The extent to which polyfluoroalkyl compounds (PFCs) are detectable in amniotic fluid is unknown. Using paired samples from 28 women, we compared the concentration of 8 PFCs measured in serum, the standard matrix for assessing human exposure, amniotic

fluid from routine amniocentesis, and urine. Perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorooctane sulfonate (PFOS), and perfluorohexane sulfonate (PFHxS) were detected in all maternal serum samples. The number of amniotic fluid samples with detectable concentrations differed by PFC (PFOA n=24; PFNA n=10; PFOS n=9; PFHxS n=4). The correlation coefficient between maternal serum and amniotic PFC levels varied considerably by PFC (PFOA rho=0.64, p<0.001; PFNA rho=0.05, p=0.9; PFOS rho=0.76, p=0.01; PFHxS rho=0.80, p=0.2). Using linear regression, PFOA appeared to be commonly detected in amniotic fluid if the serum concentration exceeded approximately 1.5 ng/mL whereas PFOS was rarely detected in amniotic fluid until the serum concentration was about 5.5 ng/mL. No PFCs were detected in urine.

Phthalates and perfluorooctanesulfonic acid in human amniotic fluid: temporal trends and timing of amniocentesis in pregnancy.
Jensen M. S., Norgaard-Pedersen B., Toft G., Hougaard D. M., Bonde J. P., Cohen A., Thulstrup A. M., Ivell R., Anand-Ivell R., Lindh C. H. and Jonsson B. A.
Environ Health Perspect. 2012;120(6):897-903.

BACKGROUND: Measures of prenatal environmental exposures are important, and amniotic fluid levels may directly reflect fetal exposures during hypothesized windows of vulnerability. OBJECTIVES: We aimed to detect various phthalate metabolites and perfluorooctanesulfonic acid (PFOS) in human amniotic fluid, to study temporal exposure trends, and to estimate potential associations with gestational week of amniocentesis and maternal age and parity at amniocentesis. METHODS: We studied 300 randomly selected second-trimester amniotic fluid samples from a Danish pregnancy-screening biobank covering 1980 through 1996. We used only samples from male offspring pregnancies. We assayed the environmental pollutants by liquid chromatography/triple quadrupole mass spectrometry and analyzed data using generalized linear regression models. RESULTS: We detected the di(2-ethylhexyl) phthalate (DEHP) metabolite mono(2-ethyl-5-carboxypentyl) phthalate (5cx-MEPP) at a median concentration of 0.27 ng/mL [interquartile range (IQR): 0.20-0.37 ng/mL], the diisononyl phthalate (DiNP) metabolite mono(4-methyl-7-carboxyheptyl) phthalate (7cx-MMeHP) at 0.07 ng/mL (IQR: 0.05-0.11 ng/mL), and PFOS at 1.1 ng/mL (IQR: 0.66-1.60 ng/mL). An increase of 1 calendar year was associated with 3.5% lower [95% confidence interval (CI): -4.8%, -2.1%] 5cx-MEPP levels and with 7.1% higher (95% CI: 5.3%, 9.0%) 7cx-MMeHP levels. For each later gestational week of amniocentesis, 5cx-MEPP was 9.9% higher (95% CI: 4.8%, 15.2%), 7cx-MMeHP was 8.6% higher (95: CI: 2.7%, 14.9%), and PFOS was 9.4% higher (95: CI: 3.3%, 15.9%). We observed no associations with maternal age or parity. CONCLUSIONS: Measured metabolite levels

appeared to parallel decreasing DEHP exposure and increasing DiNP exposure during the study period. The environmental pollutant levels were positively associated with later gestational age at amniocentesis during pregnancy weeks 12-22.

Placental transfer of perfluorinated compounds is selective--a Norwegian Mother and Child sub-cohort study.
Gutzkow K. B., Haug L. S., Thomsen C., Sabaredzovic A., Becher G. and Brunborg G.
Int J Hyg Environ Health. 2012;215(2):216-9.

Perfluorinated compounds (PFCs) comprise a large group of man-made fluorinated chemicals used in a number of consumer products and industrial applications. PFCs have shown to be persistent, bio-accumulative and widespread in the environment. Animal studies have demonstrated hepatotoxicity, immunotoxicity, developmental toxicity as well as hormonal effects. We investigated prenatal exposure to several PFCs and detected up to seven different PFCs in 123 paired samples of human maternal and cord blood, from a subcohort of the Norwegian Mother and Child Cohort Study (MoBa). The maternal and foetal levels were significantly correlated for all PFCs tested with median PFC concentrations in cord blood ranging between 30 and 79% of the maternal concentrations, demonstrating placental passage. The composition of the different PFCs varied between cord and maternal blood, with a higher proportion of shorter chained PFCs together with a higher amount of the branched isomers of perfluorooctane sulfonate (PFOS) in cord blood. Additionally, the sulfonate group seems to impede transfer efficiency. This indicates a selective placental passage of the different PFCs and hence a specific foetal exposure.

Changes in thyroid peroxidase activity in response to various chemicals.
Song M., Kim Y. J., Park Y. K. and Ryu J. C.
J Environ Monit. 2012;14(8):2121-6.

Thyroperoxidase (TPO) is a large heme-containing glycoprotein that catalyzes the transfer of iodine to thyroglobulin during thyroid hormone (TH) synthesis. Previously, we established an in vitro assay for TPO activity based on human recombinant TPO (hrTPO) stably transfected into human follicular thyroid carcinoma (FTC-238) cells. It is important to determine whether environmental chemicals can disrupt TPO activity because it is an important factor in the TH axis. In this study, we used our assay to examine the changes in TPO activity in response to various chemicals, including benzophenones (BPs), polycyclic aromatic hydrocarbons (PAHs), and persistent organic pollutants (POPs). Overall, BPs, PAHs, and POPs slightly altered TPO activity at low doses, as compared with the positive controls methimazole (MMI), genistein, and

2,2',4,4'-tetrahydroxy BP. Benzophenone, benzhydrol, 3-methylchloranthracene, pyrene, benzo(k)fluoranthene, benzo(e)pyrene, perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and heptachlor decreased TPO activity, while 2,4-dihydroxy BP, 2,2'-dihydroxy-4-methoxy BP, and dibenzo(a,h)anthracene increased TPO activity. From these data, we can predict the disruption of TPO activity by various chemicals as a sensitive TH end point. TPO activity should be considered when enacting measures to regulate environmental exposure to thyroid-disrupting chemicals.

Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996-2010. Glynn A., Berger U., Bignert A., Ullah S., Aune M., Lignell S. and Darnerud P. O. Environ Sci Technol. 2012;46(16):9071-9.

We investigated temporal trends of blood serum levels of 13 perfluorinated alkyl acids (PFAAs) and perfluorooctane sulfonamide (FOSA) in primiparous women (N = 413) from Uppsala County, Sweden, sampled 3 weeks after delivery 1996-2010. Levels of the short-chain perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) increased 11%/y and 8.3%/y, respectively, and levels of the long-chain perfluorononanoate (PFNA) and perfluorodecanoate (PFDA) increased 4.3%/y and 3.8%/y, respectively. Concomitantly, levels of FOSA (22%/y), perfluorooctane sulfonate (PFOS, 8.4%/y), perfluorodecane sulfonate (PFDS, 10%/y), and perfluorooctanoate (PFOA, 3.1%/y) decreased. Thus, one or several sources of exposure to the latter compounds have been reduced or eliminated, whereas exposure to the former compounds has recently increased. We explored if maternal levels of PFOS, PFOA, and PFNA during the early nursing period are representative for the fetal development period, using serial maternal serum samples, including cord blood (N = 19). PFAA levels in maternal serum sampled during pregnancy and the nursing period as well as in cord blood were strongly correlated. Strongest correlations between cord blood levels and maternal levels were observed for maternal serum sampled shortly before or after the delivery (r = 0.70-0.89 for PFOS and PFOA). A similar pattern was observed for PFNA, although the correlations were less strong due to levels close to the method detection limit in cord blood.

Development of an analytical strategy based on liquid chromatography-high resolution mass spectrometry for measuring perfluorinated compounds in human breast milk: application to the generation of preliminary data regarding perinatal exposure in France.

Kadar H., Veyrand B., Barbarossa A., Pagliuca G., Legrand A., Bosher C., Boquien C. Y., Durand S., Monteau F., Antignac J. P. and Le Bizec B.
Chemosphere. 2011;85(3):473-80.

Perfluorinated compounds (PFCs) are man-made chemicals for which endocrine disrupting properties and related possible side effects on human health have been reported, particularly in the case of an exposure during the early stages of development, (notably the perinatal period). Existing analytical methods dedicated to PFCs monitoring in food and/or human fluids are currently based on liquid chromatography coupled to tandem mass spectrometry, and were recently demonstrated to present some limitations in terms of sensitivity and/or specificity. An alternative strategy dedicated to the analysis of fourteen PFCs in human breast milk was proposed, based on an effective sample preparation followed by a liquid chromatography coupled to high resolution mass spectrometry measurement (LC-HRMS). This methodology confirmed the high interest for HRMS after negative ionization for such halogenated substances, and finally permitted to reach detection limits around the pg mL(-1) range with an outstanding signal specificity compared to LC-MS/MS. The proposed method was applied to a first set of 30 breast milk samples from French women. The main PFCs detected in all these samples were PFOS and PFOA with respective median values of 74 (range from 24 to 171) and 57 (range from 18 to 102) pg mL(-1), respectively. These exposure data appeared in the same range as other reported values for European countries.

Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples: manufacturing sources and transplacental transfer.
Beesoon S., Webster G. M., Shoeib M., Harner T., Benskin J. P. and Martin J. W.
Environ Health Perspect. 2011;119(11):1659-64.

BACKGROUND: Perfluorochemicals (PFCs) are detectable in the general population and in the human environment, including house dust. Sources are not well characterized, but isomer patterns should enable differentiation of historical and contemporary manufacturing sources. Isomer-specific maternal-fetal transfer of PFCs has not been examined despite known developmental toxicity of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in rodents. OBJECTIVES: We

elucidated relative contributions of electrochemical (phased out in 2001) and telomer (contemporary) PFCs in dust and measured how transplacental transfer efficiency (TTE; based on a comparison of maternal and cord sera concentrations) is affected by perfluorinated chain length and isomer branching pattern. METHODS: We analyzed matching samples of house dust (n = 18), maternal sera (n = 20), and umbilical cord sera (n = 20) by isomer-specific high-performance liquid chromatography tandem mass spectrometry. RESULTS: PFOA isomer signatures revealed that telomer sources accounted for 0-95% of total PFOA in house dust (median, 31%). This may partly explain why serum PFOA concentrations are not declining in some countries despite the phase-out of electrochemical PFOA. TTE data indicate that total branched isomers crossed the placenta more efficiently than did linear isomers for both PFOS ($p < 0.01$) and PFOA ($p = 0.02$) and that placental transfer of branched isomers of PFOS increased as the branching point moved closer to the sulfonate (SO3-) end of the molecule. CONCLUSIONS: Results suggest that humans are exposed to telomer PFOA, but larger studies that also account for dietary sources should be conducted. The exposure profile of PFOS and PFOA isomers can differ between the mother and fetus-an important consideration for perinatal epidemiology studies of PFCs.

Comparison on gestation and lactation exposure of perfluorinated compounds for newborns.
Liu J., Li J., Liu Y., Chan H. M., Zhao Y., Cai Z. and Wu Y.
Environ Int. 2011;37(7):1206-12.

Perfluorinated compounds (PFCs) are worldwide present in the environment and the general population. Animal studies have shown developmental toxicity of these compounds. To investigate the PFCs exposure of neonates from mother during gestation and lactation, we analyzed twelve PFCs in matched maternal serum, cord serum and breast milk samples collected from 50 pairs of women and their newborns between June and July 2009 in Jinhu, China. Eight PFCs were detected in serum samples, and five of them were also detectable in breast milk. A significant intercorrelation between PFCs concentrations in matched maternal serum, cord serum and breast milk was observed ($p<0.01$, $r=0.435$-0.911). The median partition ratio was from 0.39:1 (PFDA) to 1.74:1 (PFTrDA) for seven PFCs through the placenta, and was from 0.02:1 (PFOS) to 0.09:1 (PFOA) for five PFCs through the lactation. A high transport efficiency of PFOA both through placental barrier and lactation was observed. The postnatal exposure of PFCs through lactation was higher compared to prenatal exposure, especially for PFOA.

Perfluorinated chemicals in blood of residents in Wenzhou, China.

Zhang W., Lin Z., Hu M., Wang X., Lian Q., Lin K., Dong Q. and Huang C.
Ecotoxicol Environ Saf. 2011;74(6):1787-93.

Perfluorinated compounds (PFCs) are persistent organic pollutants ubiquitously distributed in the environment and human populations. Here we report PFC concentrations in the residents of Wenzhou City, which is characterized as the 'Footwear Capital' of China. Specifically, fifty serum samples collected from workers in a leather factory, fifty-five umbilical cord serum samples and fifteen serum samples from infertile men were analyzed. PFOS was one of the most frequently detected PFCs and showed the highest level. The mean serum levels of PFOS and PFOA of workers and infertile males were higher than the cord serum. PFOS concentration in cord serum increased with increase in age of the mother. Gender differences were significant both in worker serum samples and umbilical cord samples with higher levels found in males/male fetuses. Our findings suggested that PFOS, PFOA and PFHxS were widely distributed in Wenzhou residents, but occupational exposure was not the main source for workers.

Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures.
Kim S. K., Lee K. T., Kang C. S., Tao L., Kannan K., Kim K. R., Kim C. K., Lee J. S., Park P. S., Yoo Y. W., Ha J. Y., Shin Y. S. and Lee J. H.
Environ Pollut. 2011;159(1):169-74.

The levels of six perfluorocarboxylates (PFCAs), four perfloroalkylsulfonates (PFASs), and one sulfonamide were measured in paired samples of maternal serum, umbilical cord serum, and breast milk. The maternal and cord sera were strongly correlated with each other for all measured compounds ($r>0.5$ and $p<0.01$). Nevertheless, there was a significant difference in compound composition profile between the two sera matrices, with a more depletion of the longer chain compounds in cord serum. The transfer efficiency values from maternal to cord serum (TFCS/MS) decreased by 70% with each increasing unit of -CF2 chain within a PFCA group, and for perfluorooctanesulfonate (PFOS), by a half compared to perfluorooctanoate (PFOA). In contrast to the strong correlation in concentrations between the two sera matrices, the pattern of compounds in breast milk differed considerably with those in sera. Accordingly, compound- and matrix-specific transfer must be considered when assessing prenatal and postnatal exposure.

A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden.
Sundstrom M., Ehresman D. J., Bignert A., Butenhoff J. L., Olsen G. W., Chang S. C. and Bergman A.
Environ Int. 2011;37(1):178-83.

The widespread presence of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and perfluorohexanesulfonate (PFHxS) in human general populations and their slow elimination profiles have led to renewed interest in understanding the potential human neonatal exposures of perfluoroalkyls (PFAs) from consumption of human milk. The objective of this study was to evaluate the concentrations of PFOS, PFHxS, and PFOA in pooled human milk samples obtained in Sweden between 1972 and 2008 (a period representing the most significant period of PFA production) and to see whether the time trend of these analytes parallels that indicated in human serum. Chemical analysis of PFOS, PFHxS, and PFOA was performed on pooled Swedish human milk samples from 1972 to 2008 after methodological refinements. The 20 samples which formed the 2007 pool were also analyzed individually to evaluate sample variations. Analyses were performed by HPLC-MS/MS. Due to the complexities of the human milk matrix and the requirement to accurately quantitate low pg/mL concentrations, meticulous attention must be paid to background contamination if accurate results are to be obtained. PFOS was the predominant analyte present in the pools and all three analytes showed statistically significant increasing trends from 1972 to 2000, with concentrations reaching a plateau in the 1990s. PFOA and PFOS showed statistically significant decreasing trends during 2001-2008. At the end of the study, in 2008, the measured concentrations of PFOS, PFHxS, and PFOA in pooled human milk were 75 pg/mL, 14 pg/mL, and 74 pg/mL, respectively. The temporal concentration trends of PFOS, PFHxS, and PFOA observed in human milk are parallel to those reported in the general population serum concentrations.

Analysis of perfluorinated chemicals in umbilical cord blood by ultra-high performance liquid chromatography/tandem mass spectrometry.
Lien G. W., Wen T. W., Hsieh W. S., Wu K. Y., Chen C. Y. and Chen P. C.
J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(9-10):641-6.

Perfluorinated compounds (PFCs) can cross the placental barrier and enter fetal circulation. This study aimed at developing a fast and sensitive ultra-high performance liquid chromatography/tandem mass spectrometry method for the determination of twelve perfluorinated compounds in cord blood. Samples were processed with protein

precipitation using formic acid and methanol, mixed with stable isotope labeled standard, followed by sonication and centrifugation, and were analyzed using a Waters ACQUITY UPLC coupled with a Waters Quattro Premier XE triple-quadrupole mass spectrometer. The instrument was operated in selected reaction monitoring (SRM) with negative electrospray ionization. Using BEH C(18) column (2.1 mmx50 mm, 1.7 mum) with 10-mM N-methylmorpholine/methanol gradient elution provided a fast chromatographic separation (5.5 min) and sharp peaks. Intra- and inter-day calibration bias was less than 7% and intra- and inter-day calibration of relative standard deviations were within 0.02-8.22% for all the analytes and concentrations. The recoveries of PFCs spiked into bovine serum ranged from 85 to 104% with relative standard deviations from 0.02 to 6.37%. The limits of quantitation (LOQs), defined as a signal-to-noise ratio of ten, ranged from 0.15 to 3.1 ng/mL for the twelve PFCs. Perfluorooctanoic acid (PFOA), perfluorooctyl sulfonate (PFOS), perfluoroundecanoic acid (PFUA) and perfluorononanoic acid (PFNA) were detected in up to 68% of umbilical cord plasma (n=444) in Taiwan Birth Panel Study and the health effect of these chemicals on children developmental deserves further investigation.

[Occurrence and relevance to health of persistent organic substances and phthalates in breast milk].
Fromme H., Raab U., Furst P., Vieth B., Volkel W., Albrecht M. and Schwegler U. Gesundheitswesen. 2011;73(1):e27-43.

The aim of this study is to give an overview of the concentrations of persistent organic pollutants like the polychlorinated dibenzo- P-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB), polybrominated diphenyl ether (PBDE), perfluorinated compounds (PFC) and of phthalates in breast milk. On the basis of median and 95th percentile values an "average" and a "high" intake were calculated for a 3-month-old infant exclusively breast-fed. Moreover, the actual daily intake was compared with tolerable daily intakes (TDI) recommended by scientific institutions. On this basis, we found an "average" ("high") daily intake of 70 (140) pg TEQ/kg body weight (b. w.) for PCDD/F and dioxin-like PCB (dl-PCB), 10 (20) ng/kg b. w. for PFOS (perfluorooctanesulfonate), 20 (50) ng/kg b. w. for PFOA (perfluorooctanoate), 1.7 (7.5) ng/kg b. w. for BDE 47, and 0.6 (2.1) ng/kg b. w. for BDE 99. For di-2-ethylhexyl phthalate (DEHP) and di- N-butyl phthalate (DnBP) an "average" and "high" intake of 400 ng/kg b. w. and 2,000 ng/kg b. w. and of 100 and 500 ng/kg b.w. were assumed, respectively. For all of these substances we found a daily intake via breast milk below the TDI, established on a livelong basis. On contrary, the daily intake for the sum of the PCDD/F and dl-PCB considerably exceeded the recommended TDI value. Even with regard to the "high" daily intake values the share of PBDE, PFC, and phthalates on the

TDI was only in the lower percentage. Scientific organisations assume that an exceeding of the PCDD/F and dl-PCB intake in relation to the TDI value is acceptable only on the basis of the still declining levels in breast milk and the fact that this high exposure only occurs during some months of the entire life when breast milk is consumed. On the basis of the recent exposure situation mothers can exclusively breast-feed their infants for 6 months without any hesitation. The well established health benefits for mothers and infants when exclusively breast-feeding should be utilised. There is also no health concern if the mother decides to breast-feed the baby for longer than 6 months when the infant also receives additional food.

Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation.
Thomsen C., Haug L. S., Stigum H., Froshaug M., Broadwell S. L. and Becher G. Environ Sci Technol. 2010;44(24):9550-6.

At present, scientific knowledge on depuration rates of persistent organic pollutants (POPs) is limited and the previous assumptions of considerable reduction of body burdens through breast-feeding have recently been challenged. We therefore studied elimination rates of important POPs in nine Norwegian primiparous mothers and one mother breast-feeding her second child by collecting breast-milk samples (n = 70) monthly from about two weeks to up to twelve months after birth. Perfluorinated compounds (PFCs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), and polychlorinated biphenyls (PCBs) were determined in the breast-milk samples. Linear mixed effect models were established for selected compounds, and significant decreases in the range of 1.2-4.7% in breast-milk concentrations per month were observed for a wide range of PCBs and PBDEs. For the first time, depuration rates for perfluorooctylsulfonate (PFOS) and perfluorooctanoic acid (PFOA) are presented, being 3.8 and 7.8% per month, respectively ($p < 0.05$). The relative amount of the branched PFOS isomers in the breast-milk samples was 18% on average (range 6-36%, RSD 30%). There were no significant differences in isomer pattern between the mothers, or changes during the lactation period. After a year of nursing the breast-milk concentrations of PFCs, PBDEs, and PCBs were reduced by 15-94%.

Pre- and postnatal exposure to perfluorinated compounds (PFCs).
Fromme H., Mosch C., Morovitz M., Alba-Alejandre I., Boehmer S., Kiranoglu M., Faber F., Hannibal I., Genzel-Boroviczeny O., Koletzko B. and Volkel W.
Environ Sci Technol. 2010;44(18):7123-9.

Perfluorinated compounds (PFCs) are a group of chemicals widely used for many applications. In this study PFCs were investigated in maternal blood during pregnancy (at two time points) (n = 40 and 38) and 6 months after delivery (n = 47), in cord blood (n = 33) and in blood of infants six (n = 40) and nineteen months (n = 24) after birth, and monthly in breast milk samples in Germany. Concentrations in maternal serum ranged from 0.5 to 9.4 mug/L for perfluorooctane sulfonate (PFOS) and 0.7 to 8.7 mug/L for perfluorooctanoic acid (PFOA). In cord serum, the values ranged from 0.3 to 2.8 mug/L and from 0.5 to 4.2 mug/L for PFOS and PFOA, respectively. The median results from serum at six and nineteen months of age were 3.0 and 1.9 mug/L for PFOS and 6.9 and 4.6 mug/L for PFOA, respectively. In breast milk samples, PFOS ranged from <0.03 to 0.11 mug/L (median: 0.04 mug/L), while PFOA was detected only in some samples as were all other PFCs. Overall, we found low levels of PFCs in cord sera and an increase in concentrations through the first months of infant life. Although the concentrations in breast milk were low, this intake led to a body burden at the age of six months similar to (PFOS) or higher than (PFOA) that found in adults.

Brominated flame retardants and perfluorinated chemicals, two groups of persistent contaminants in Belgian human blood and milk.
Roosens L., D'Hollander W., Bervoets L., Reynders H., Van Campenhout K., Cornelis C., Van Den Heuvel R., Koppen G. and Covaci A.
Environ Pollut. 2010;158(8):2546-52.

We assessed the exposure of the Flemish population to brominated flame retardants (BFRs) and perfluorinated compounds (PFCs) by analysis of pooled cord blood, adolescent and adult serum, and human milk. Levels of polybrominated diphenyl ethers (PBDEs) in blood (range 1.6-6.5 ng/g lipid weight, lw) and milk (range 2.0-6.4 ng/g lw) agreed with European data. Hexabromocyclododecane ranged between <2.1-5.7 ng/g lw in milk. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) dominated in blood and ranged between 1 and 171 ng/mL and <0.9-9.5 ng/mL, respectively. Total PFC levels in milk ranged between <0.5-29 ng/mL. A significant increase in PBDE concentrations was detected from newborns (median 2.1) to the adolescents and adults (medians 3.8 and 4.6 ng/g lw, respectively). An identical trend

was observed for PFOS, but not for PFOA. We estimated that newborn exposure to BFRs and PFCs occurs predominantly post-natally, whereas placental transfer has a minor impact on the body burden.

Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds.
Guerrero-Preston R., Goldman L. R., Brebi-Mieville P., Ili-Gangas C., Lebron C., Witter F. R., Apelberg B. J., Hernandez-Roystacher M., Jaffe A., Halden R. U. and Sidransky D.
Epigenetics. 2010;5(6):539-46.

Environmental exposures in-utero may alter the epigenome, thus impacting chromosomal stability and gene expression. We hypothesized that in utero exposures to maternal smoking and perfluoroalkyl compounds (PFCs) are associated with global DNA hypomethylation in umbilical cord serum. Our objective was to determine if global DNA methylation could be used as a biomarker of in utero exposures to maternal smoking and PFCs. Using an ELISA-based method, global DNA methylation was quantified in umbilical cord serum from 30 newborns with high (> 10 ng/ml, mean 123.8 ng/ml), low (range 1-10 ng/ml, mean 1.6 ng/ml) and very low (< 1 ng/ml, mean 0.06 ng/ml) cord serum cotinine levels. Y chromosome analysis was performed to rule out maternal DNA cross-contamination. Cord serum global DNA methylation showed an inverse dose response to serum cotinine levels ($p< 0.001$). Global DNA methylation levels in cord blood were the lowest among newborns with smoking mothers (mean=15.04%; 95% CI, 8.4, 21.7) when compared to babies of mothers who were second-hand smokers (21.1%; 95% CI, 16.6, 25.5) and non-smokers (mean=29.2%; 95% CI, 20.1, 38.1). Global DNA methylation was inversely correlated with serum PFOA ($r= -0.72$, $p < 0.01$) but not PFOS levels. Serum Y chromosome analyses did not detect maternal DNA cross-contamination. This study supports the use of global DNA methylation status as a biomarker of in utero exposure to cigarette smoke and PFCs.

Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study.
Hanssen L., Rollin H., Odland J. O., Moe M. K. and Sandanger T. M.
J Environ Monit. 2010;12(6):1355-61.

There is limited information about both environmental and human perfluorinated compounds (PFCs) concentrations in the southern hemisphere, and for the first time, concentrations of these compounds are reported in maternal serum and cord blood of South African women. The majority of the participants were of African Black ethnicity,

with a similar socioeconomic status. In maternal serum perfluorooctane sulfonate (PFOS) was found to be the most abundant PFC (1.6 ng mL(-1)), followed by perfluorooctanoate (PFOA: 1.3 ng mL(-1)) and perfluorohexane sulfonate (PFHxS: 0.5 ng mL(-1)); however, in cord blood PFOA was the most abundant compound (1.3 ng mL(-1)) followed by PFOS (0.7 ng mL(-1)) and PFHxS (0.3 ng mL(-1)). Linear PFOS constituted 58% of the sum of PFOS, comparable with a reported percentage from Australia. Differences in PFC concentrations between communities were found, with the highest concentrations in urban and semi-urban areas. The median maternal PFOS concentration was lower than has been reported in other studies, whereas the PFOA concentration was the same. This clearly indicates that the exposure pathway is different from the western world. Significant differences in housing quality were observed and the urban and sub-urban community had the highest living and housing standards. Possible exposure pathways could be different from those elucidated in the western world with the exception of the urban community in our study that showed higher living standards in general and easier access to modern consumer products.

Perfluorinated compounds in delivering women from south central Vietnam.
Rylander C., Phi D. T., Odland J. O. and Sandanger T. M.
J Environ Monit. 2009;11(11):2002-8.

The associations between age, body mass index (BMI), parity, place of residence (coastal or inland) and plasma concentrations of perfluorinated compounds (PFCs) were assessed in a study population from south central Vietnam. The study group consisted of 91 delivering women of varied age (18-40 years) from two different locations (37 urban, 36 rural and 18 with unknown residence). Perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulfonate (PFHxS) were detected in 98-100% of all samples. PFOS (median 3.2 ng/ml) was the most common compound followed by PFOA (median 1.6 ng/ml), PFHxS (median 0.7 ng/ml) and perfluorononanoate (PFNA) (median 0.7 ng/ml). The women from the coastal city Nha Trang had higher concentrations of all investigated compounds than women from the inland district Dien Khanh. The two study locations are situated only 10 km apart and the diet is considered somewhat similar, however, women in Dien Khanh are more self-sufficient with locally produced food. The family income in Nha Trang is also most likely higher than in Dien Khanh and this may affect living conditions, e.g. quality of housing, which in turn may influence the exposure to PFCs. There were no associations between age, parity or BMI and the investigated PFCs. Linear PFOS constituted 83% of the sum of PFOS. This is considerably higher than reported in other studies from Europe and Australia and may indicate differences in exposure sources between countries, or differences in exposure time and persistency of the different isomers.

Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin.

Weiss J. M., Andersson P. L., Lamoree M. H., Leonards P. E., van Leeuwen S. P. and Hamers T.

Toxicol Sci. 2009;109(2):206-16.

Due to their unique surfactant properties, poly- and perfluorinated compounds (PFCs) have been extensively used and can be found all over the environment. Concern about their environmental fate and toxicological properties has initiated several research projects. In the present study, we investigated if PFCs can compete with thyroxine (T(4), i.e., the transport form of thyroid hormone) for binding to the human thyroid hormone transport protein transthyretin (TTR). Such competitive capacity may lead to decreased thyroid hormone levels as previously reported for animals exposed to PFCs. Twenty-four PFCs, together with 6 structurally similar natural fatty acids, were tested for binding capacity in a radioligand-binding assay. The binding potency decreased in the order: perfluorohexane sulfonate > perfluorooctane sulfonate/perfluorooctanoic acid > perfluoroheptanoic acid > sodium perfluoro-1-octanesulfinate > perfluorononanoic acid, with TTR binding potencies 12.5-50 times lower than the natural ligand T(4). Some lower molecular weight compounds with structural similarity to these PFCs were > 100 times less potent than T(4). Simple descriptors based on the two-dimensional molecular structures of the compounds were used to visualize the chemical variation and to model the structure-activity relationship for the competitive potencies of the TTR-binding compounds. The models indicated the dependence on molecular size and functional groups but demanded a more detailed description of the chemical properties and data for validation and further quantitative structure-activity relationship (QSAR) development. Competitive binding of PFCs to TTR, as observed for human TTR in the present study, may explain altered thyroid hormone levels described for PFC-exposed rats and monkeys. Median human blood levels of the most potent TTR-binding PFCs are one to two orders of magnitude lower than concentration at 50% inhibition (IC(50)) values determined in the present study. In addition, this study contributes to the understanding of the bioaccumulation of PFCs in man and possibly in other wildlife species.

Polyfluoroalkyl chemicals in the serum and milk of breastfeeding women.
von Ehrenstein O. S., Fenton S. E., Kato K., Kuklenyik Z., Calafat A. M. and Hines E. P.
Reprod Toxicol. 2009;27(3-4):239-45.

Polyfluoroalkyl chemicals (PFCs) comprise a group of man-made organic compounds, some of which are persistent contaminants with developmental toxicity shown in laboratory animals. There is a paucity of human perinatal exposure data. The US EPA conducted a pilot study (Methods Advancement for Milk Analysis) including 34 breastfeeding women in North Carolina. Milk and serum samples were collected at 2-7 weeks and 3-4 months postpartum; 9 PFCs were assessed in milk and 7 in serum. Perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) were found in nearly 100% of the serum samples. PFOS and PFOA were found at the highest concentrations. PFCs were below the limit of quantification in most milk samples. Serum concentrations of PFOS, PFOA and PFHxS were lower ($p<0.01$) at the second visit compared to the first visit. Living in North Carolina 10 years or longer was related to elevated PFOS, PFOA and PFNA ($p<or=0.03$). These pilot data support the need to further explore perinatal PFC exposures and potentially related health effects, as planned in the upcoming National Children's Study which provided the framework for this investigation.

Perfluorinated compounds in delivering women from south central Vietnam.
Rylander C., Phi D. T., Odland J. O. and Sandanger T. M.
J Environ Monit. 2009;11(11):2002-8.

The associations between age, body mass index (BMI), parity, place of residence (coastal or inland) and plasma concentrations of perfluorinated compounds (PFCs) were assessed in a study population from south central Vietnam. The study group consisted of 91 delivering women of varied age (18-40 years) from two different locations (37 urban, 36 rural and 18 with unknown residence). Perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA) and perfluorohexane sulfonate (PFHxS) were detected in 98-100% of all samples. PFOS (median 3.2 ng/ml) was the most common compound followed by PFOA (median 1.6 ng/ml), PFHxS (median 0.7 ng/ml) and perfluorononanoate (PFNA) (median 0.7 ng/ml). The women from the coastal city Nha Trang had higher concentrations of all investigated compounds than women from the inland district Dien Khanh. The two study locations are situated only 10 km apart and the diet is considered somewhat similar, however, women in Dien Khanh are more self-sufficient with locally produced food. The family income in Nha Trang is also most likely higher than in Dien Khanh and this may affect living conditions, e.g. quality of

housing, which in turn may influence the exposure to PFCs. There were no associations between age, parity or BMI and the investigated PFCs. Linear PFOS constituted 83% of the sum of PFOS. This is considerably higher than reported in other studies from Europe and Australia and may indicate differences in exposure sources between countries, or differences in exposure time and persistency of the different isomers.

Analysis of blood spots for polyfluoroalkyl chemicals.
Kato K., Wanigatunga A. A., Needham L. L. and Calafat A. M.
Anal Chim Acta. 2009;656(1-2):51-5.

Polyfluoroalkyl chemicals (PFCs) have been detected in humans, in the environment, and in ecosystems around the world. The potential for developmental and reproductive toxicities of some PFCs is of concern especially to children's health. In the United States, a sample of a baby's blood, called a "dried blood spot" (DBS), is obtained from a heel stick within 48 h of a child's birth. DBS could be useful for assessing prenatal exposure to PFCs. We developed a method based on online solid phase extraction coupled with high performance liquid chromatography-isotope dilution tandem mass spectrometry for measuring four PFCs in DBS, perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate, perfluorooctanoate (PFOA), and perfluorononanoate. The analytical limits of detection using one whole DBS (approximately 75 microL of blood) were <0.5 ng mL(-1). To validate the method, we analyzed 98 DBS collected in May 2007 in the United States. PFOS and PFOA were detected in all DBS at concentrations in the low ng mL(-1) range. These data suggest that DBS may be a suitable matrix for assessing perinatal exposure to PFCs, but additional information related to sampling and specimen storage is needed to demonstrate the utility of these measures for assessing exposure.

Perfluorinated compounds in human milk from Massachusetts, U.S.A.
Tao L., Kannan K., Wong C. M., Arcaro K. F. and Butenhoff J. L.
Environ Sci Technol. 2008;42(8):3096-101.

Perfluorinated compounds (PFCs), notably perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA), have been reported in human blood. Furthermore, the occurrence of PFCs in the blood of newborn babies, coupled with the need to study the potential association of PFC exposure with birth outcomes in neonates, suggests the need for determining the sources and magnitude of exposure in infants. In this study, nine PFCs were measured in 45 human breast milk samples collected in 2004 from Massachusetts, U.S.A. PFOS and PFOA were the predominant PFCs found at mean concentrations of 131 and 43.8 pg/mL, respectively. Comparison of the ratio of PFOS to

PFOA in human milk with the ratios published for human serum from the U.S. female population suggested preferential partitioning of PFOA to milk. Concentrations of PFOA were significantly higher in the milk of mothers nursing for the first time (n = 34) than in the milk of mothers who have previously nursed (n = 8). Based on the estimated body weight and milk intake, the average and highest daily intakes of total PFCs by infants were 23.5 and 87.1 ng/kg bw, respectively. We found that the daily ingestion rates of PFOS and PFOA did not exceed the tolerable daily intake recommended by the U.K. Food Standards Agency. This is the first study to measure the occurrence of PFCs in human milk from the U.S.A.

Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States.
Tao L., Ma J., Kunisue T., Libelo E. L., Tanabe S. and Kannan K.
Environ Sci Technol. 2008;42(22):8597-602.

The occurrence of perfluorinated compounds (PFCs) in human blood is known to be widespread; nevertheless, the sources of exposure to humans, including infants, are not well understood. In this study, breast milk collected from seven countries in Asia was analyzed (n=184) for nine PFCs, including perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). In addition, five brands of infant formula (n=21) and 11 brands of dairy milk (n=12) collected from retail stores in the United States were analyzed, for comparison with PFC concentrations previously reported for breast milk from the U.S. PFOS was the predominant PFC detected in almost all Asian breast milk samples, followed by perfluorohexanesulfonate (PFHxS) and PFOA. Median concentrations of PFOS in breast milk from Asian countries varied significantly;the lowest concentration of 39.4 pg/mL was found in India, and the highest concentration of 196 pg/mL was found in Japan. The measured concentrations were similarto or less than the concentrations previously reported from Sweden, the United States, and Germany (median, 106-166 pg/mL). PFHxS was found in more than 70% of the samples analyzed from Japan, Malaysia, Philippines, and Vietnam, at mean concentrations ranging from 6.45 (Malaysia) to 15.8 (Philippines) pg/mL PFOA was found frequently only in samples from Japan; the mean concentration for that country was 77.7 pg/mL. None of the PFCs were detected in the infant-formula or dairy-milk samples from the U.S. except a few samples that contained concentrations close to the limit of detection. The estimated average daily intake of PFOS by infants from seven Asian countries, via breastfeeding, was 11.8 +/- 10.6 ng/kg bw/ day; this value is 7-12 times higher than the estimated adult dietary intakes previously reported from Germany, Canada, and Spain. The average daily intake of PFOA by Japanese infants was 9.6 +/- 4.9 ng/kg bw/day, a value 3-10 times greater than the estimated adult dietary intakes

reported from Germany and Canada. The highest estimated daily intakes of PFOS and PFOA by infants from seven Asian countries studied were 1-2 orders of magnitude below the tolerable daily intake values recommended by the U.K. Food Standards Agency.

Use of newborn screening program blood spots for exposure assessment: declining levels of perluorinated compounds in New York State infants.
Spliethoff H. M., Tao L., Shaver S. M., Aldous K. M., Pass K. A., Kannan K. and Eadon G. A.
Environ Sci Technol. 2008;42(14):5361-7.

Temporal biomonitoring studies can assess changes in population exposures to contaminants, but collection of biological specimens with adequate representation and sufficient temporal resolution can be resource-intensive. Newborn Screening Programs (NSPs) collect blood as dried spots on filter paper from nearly all infants born in the United States (U.S.). In this study, we investigated the use of NSP blood spots for temporal biomonitoring by analyzing perfluorooctane sulfonate (PFOS), perfluorooctane sulfonamide (PFOSA), perfluorohexane sulfonate (PFHxS), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) in 110 New York State (NYS) NSP blood spot composite specimens collected between 1997 and 2007, representing a total of 2640 infants. All analytes were detected in > or =90% of the specimens. Concentrations of PFOS, PFOSA, PFHxS, and PFOA exhibited significant exponential declines after the year 2000, coinciding with the phase-out in PFOS production in the U.S. Calculated disappearance half-lives for PFOS, PFHxS, and PFOA (4.4, 8.2, and 4.1 years, respectively) were similar to biological half-lives reported for retired fluorochemical workers. Our results suggest sharp decreases in perinatal exposure of NYS infants to PFOS, PFOSA, PFHxS, and PFOA and demonstrate, for the first time, the utility of NSP blood spots for assessment of temporal trends in exposure.

Dietary predictors of perfluorinated chemicals: a study from the Danish National Birth Cohort.
Halldorsson T. I., Fei C., Olsen J., Lipworth L., McLaughlin J. K. and Olsen S. F.
Environ Sci Technol. 2008;42(23):8971-7.

This study investigated the association between dietary variables and plasma levels of perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) among 1076 pregnant women. Diet was assessed at midpregnancy by a food-frequency questionnaire. Mean first trimester plasma PFOS and PFOA levels were 35.1 and 5.6 ng/mL respectively. PFOS levels were positively associated ($p < 0.05$) with intake of red

meat, animal fats, and snacks (e.g., popcorn, potato chips), whereas intake of vegetables and poultry was inversely associated. The adjusted mean differences between the 75th and 25th intake percentiles were 4.3 ng/mL [95% CI: 2.1, 6.5] for red meat 3.4 ng/mL [95% CI: 1.2, 5.6] for animal fats, and 2.0 ng/mL [95% CI: 0.3, 3.6] for snacks. Similar but weaker associations were observed for PFOA. Furthermore, a comparison between women reporting low (< or =25th percentile) red meat and high (> or =75th percentile) vegetable intake and women reporting low vegetable and high red meat intake resulted in differences in plasma PFOS and PFOA concentrations equal to 31% and 18% of mean levels, respectively. Studies quantifying levels of perfluorinated compounds in food have suggested that diet could be an important route of human exposure. The observed associations in our study between dietary variables and maternal exposure further support that conclusion.

Determinants of fetal exposure to polyfluoroalkyl compounds in Baltimore, Maryland.
Apelberg B. J., Goldman L. R., Calafat A. M., Herbstman J. B., Kuklenyik Z., Heidler J., Needham L. L., Halden R. U. and Witter F. R.
Environ Sci Technol. 2007;41(11):3891-7.

Polyfluoroalkyl compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are ubiquitous, man-made chemicals. Human data suggest that in utero exposures to these chemicals occur and some evidence of developmental toxicity in animals exists. To assess the distribution and determinants of fetal exposure to PFCs, we analyzed cord serum samples from 299 singleton newborns delivered between 2004 and 2005 in Baltimore, MD for 10 PFCs by employing on-line solid-phase extraction coupled with reversed-phase high-performance liquid chromatography-tandem mass spectrometry. PFOS and PFOA were detected in 99 and 100% of umbilical cord sera, with geometric mean concentrations of 4.9 and 1.6 ng/mL, respectively. PFOS and PFOA concentrations were highly correlated (Pearson's r = 0.64 after natural log transformation, $p < 0.01$). Eight other PFCs were detected less frequently and at lower concentrations than PFOS and PFOA. Geometric mean concentrations of PFOS for Asians (6.0 ng/mL) and Blacks (5.1 ng/mL) were higher than those for Whites (4.2 ng/mL), while PFOA levels were more evenly distributed by race. Other maternal demographic and socioeconomic characteristics, including age, education, marital status, and living in the city limits were not significantly associated with cord concentrations. Our findings suggest that in utero exposure to PFOS and PFOA is ubiquitous in a population of babies born in Baltimore, MD.

Exposure of perfluorinated chemicals through lactation: levels of matched human milk and serum and a temporal trend, 1996-2004, in Sweden.

Karrman A., Ericson I., van Bavel B., Darnerud P. O., Aune M., Glynn A., Lignell S. and Lindstrom G.
Environ Health Perspect. 2007;115(2):226-30.

BACKGROUND: Only limited data exist on lactation as an exposure source of persistent perfluorinated chemicals (PFCs) for children. OBJECTIVES: We studied occurrence and levels of PFCs in human milk in relation to maternal serum together with the temporal trend in milk levels between 1996 and 2004 in Sweden. Matched, individual human milk and serum samples from 12 primiparous women in Sweden were analyzed together with composite milk samples (25-90 women/year) from 1996 to 2004. RESULTS: Eight PFCs were detected in the serum samples, and five of them were also above the detection limits in the milk samples. Perfluorooctanesulfonate (PFOS) and perfluorohexanesulfonate (PFHxS) were detected in all milk samples at mean concentrations of 0.201 ng/mL and 0.085 ng/mL, respectively. Perfluorooctanesulfonamide (PFOSA), perfluorooctanoic acid (PFOA), and perfluorononanoic acid (PFNA) were detected less frequently. DISCUSSION: The total PFC concentration in maternal serum was 32 ng/mL, and the corresponding milk concentration was 0.34 ng/mL. The PFOS milk level was on average 1% of the corresponding serum level. There was a strong association between increasing serum concentration and increasing milk concentration for PFOS (r(2) = 0.7) and PFHxS (r(2) = 0.8). PFOS and PFHxS levels in composite milk samples were relatively unchanged between 1996 and 2004, with a total variation of 20 and 32% coefficient of variation, respectively. CONCLUSION: The calculated total amount of PFCs transferred by lactation to a breast-fed infant in this study was approximately 200 ng/day. Lactation is a considerable source of exposure for infants, and reference concentrations for hazard assessments are needed.

Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study.
Midasch O., Drexler H., Hart N., Beckmann M. W. and Angerer J.
Int Arch Occup Environ Health. 2007;80(7):643-8.

OBJECTIVES: Perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) can be released of perfluorinated compounds by biotic and/or metabolic decomposition. Due to their ubiquitous occurrence, persistence and bioaccumulative properties they can be found in blood of the general population all over the world. In animal studies PFOS and

PFOA provoked cancer and showed developmental toxic potential besides other adverse health effects. On the basis of the comparison of maternal and umbilical cord plasma sample pairs we wanted to examine whether infants are exposed to PFOS and PFOA via their mothers' blood. METHODS: We determined PFOS and PFOA in 11 plasma samples of mothers and the 11 corresponding cord plasma samples of neonates. An analytical method based on plasma protein precipitation followed by HPLC with MS/MS-detection was employed. As internal standards we used 1,2,3,4-(13)C(4)-PFOS and 1,2-(13)C(2)-PFOA. RESULTS: We found PFOS and PFOA in every plasma sample analysed. In maternal plasma samples PFOS concentrations were consistently higher compared to those of the related cord plasma samples (median: 13.0 microg/l vs. 7.3 microg/l). In the case of PFOA we observed only minor differences between PFOA concentrations within the analysed sample pairs (median: 2.6 microg/l vs. 3.4 microg/l for maternal and cord plasma samples, respectively). DISCUSSION: For both substances a crossing of the placental barrier could be shown. For PFOS we observed a decrease from maternal to cord plasma concentrations by a factor of 0.41-0.80. To the contrary, PFOA crosses the placental barrier obviously unhindered. These findings show that neonates are exposed to PFOS and PFOA via their mothers' blood. Given the current situation that only little is known about the consequences of PFOS and PFOA exposure in the early state of development of humans and the fact that in animal studies both substances showed developmental toxic effects further research regarding human health effects is indispensable.

Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy.
Inoue K., Okada F., Ito R., Kato S., Sasaki S., Nakajima S., Uno A., Saijo Y., Sata F., Yoshimura Y., Kishi R. and Nakazawa H.
Environ Health Perspect. 2004;112(11):1204-7.

Fluorinated organic compounds (FOCs), such as perfluorooctane sulfonate (PFOS), perfluoro-octanoate (PFOA), and perfluorooctane sulfonylamide (PFOSA), are widely used in the manufacture of plastic, electronics, textile, and construction material in the apparel, leather, and upholstery industries. FOCs have been detected in human blood samples. Studies have indicated that FOCs may be detrimental to rodent development possibly by affecting thyroid hormone levels. In the present study, we determined the concentrations of FOCs in maternal and cord blood samples. Pregnant women 17-37 years of age were enrolled as subjects. FOCs in 15 pairs of maternal and cord blood samples were analyzed by liquid chromatography-electrospray mass spectrometry coupled with online extraction. The limits of quantification of PFOS, PFOA, and PFOSA

in human plasma or serum were 0.5, 0.5, and 1.0 ng/mL, respectively. The method enables the precise determination of FOCs and can be applied to the detection of FOCs in human blood samples for monitoring human exposure. PFOS concentrations in maternal samples ranged from 4.9 to 17.6 ng/mL, whereas those in fetal samples ranged from 1.6 to 5.3 ng/mL. In contrast, PFOSA was not detected in fetal or maternal samples, whereas PFOA was detected only in maternal samples (range, < 0.5 to 2.3 ng/mL, 4 of 15). Our results revealed a high correlation between PFOS concentrations in maternal and cord blood (r2 = 0.876). However, we did not find any significant correlations between PFOS concentration in maternal and cord blood samples and age bracket, birth weight, or levels of thyroid-stimulating hormone or free thyroxine. Our study revealed that human fetuses in Japan may be exposed to relatively high levels of FOCs. Further investigation is required to determine the postnatal effects of fetal exposure to FOCs. Key words: cord blood, fluorinated organic compounds, human, PFOA, PFOS, PFOSA, pregnancy.

[Status of perfluorochemicals in adult serum and umbilical blood in Shenyang].
Jin Y., Liu X., Li T., Qin H. and Zhang Y.
Wei Sheng Yan Jiu. 2004;33(4):481-3.

OBJECTIVE: To study the status of perfluorooctane sulfonate (PFOS), and Perfluorooctanoic acid (FOA) pollution in serum and umbilical blood among general people in Shenyang area. METHODS: Concentration of PFOS and PFOA in adult serum and umbilical blood samples was measured by means of liquid phase chromatography/mass spectrograph selective iron monitoring (PFOS: m/z = 499, PFOA: m/z = 413). RESULTS: It was showed that geometric mean of serum concentration of PFOS of male was 40.73microg/L and that of female was 45.46microg/L, PFOA is 11.53microg/L and 8.97microg/L. Geometric mean concentration of PFOS and PFOA in umbilical blood was 2.214microg/L and 0.264microg/L. There was no correlativity between concentration of PFOS, PFOA and age in adult serum and umbilical blood. CONCLUSION: It was suggested that there was PFOS contamination in common group in Shenyang. Also, fetus was exposed in PFOS and PFOA during its embryonic period. There were also PFOS and PFOA pollution in human umbilical blood samples.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Effects of developmental perfluorooctane sulfonate exposure on spatial learning and memory ability of rats and mechanism associated with synaptic plasticity.
Wang Y., Liu W., Zhang Q., Zhao H. and Quan X.
Food Chem Toxicol. 2015;76:70-6.

The present study aims to explore the effects of perfluorooctane sulfonate (PFOS) on cognitive function in developing rats and the underlying mechanism associated with synaptic plasticity. Pregnant Wistar rats were fed with 0, 5, and 15 mg/L of PFOS via drinking water during gestation and lactation. Offspring were exposed to PFOS on prenatal and/or postnatal days by cross-fostering. Spatial learning and memory abilities were tested from postnatal day (PND) 35. We also analyzed the expression pattern of the synaptic plasticity-related genes and proteins in the hippocampus on PND7 and PND35. Results revealed that PFOS exposure reduced the spatial learning and memory abilities of the offspring, particularly of those with prenatal exposure. Meanwhile, protein levels of growth-associated protein-43, neural cell adhesion molecule 1, nerve growth factor, and brain-derived neurotrophic factor decreased on PND35, which are involved in the formation of synaptic plasticity. In contrast, significant increase in gap-43, ncam1, and bdnf genes on the mRNA level was observed on PND7, possibly due to the post-transcriptional mechanism. Results of both behavioral effects and molecular endpoints suggested the high risk of prenatal PFOS exposure. The decline of spatial learning and memory abilities induced by developmental PFOS exposure was closely related to synaptic plasticity.

Comparison of waterborne and in ovo nanoinjection exposures to assess effects of PFOS on zebrafish embryos.
Li Y., Han Z., Zheng X., Ma Z., Liu H., Giesy J. P., Xie Y. and Yu H.
Environ Sci Pollut Res Int. 2015;22(3):2303-10.

Since perfluorooctane sulfonate (PFOS) had been detected in eggs of seabirds and fish, toxicity of waterborne PFOS to embryonic development of zebrafish (Danio rerio) was investigated. However, because assessment of effects by use of dietary exposure of adults is time-consuming and expensive, a study was conducted to compare effects

on embryos via nanoinjection and waterborne exposure. Nanoinjection, in which small amounts of chemicals are injected into developing eggs, was used to incorporate PFOS into the yolk sac of embryos of zebrafish. Effects of PFOS during the period of development of the embryo were assessed within 96 h post-fertilization (hpf). PFOS significantly retarded development of embryos of zebrafish and resulted in abnormalities as well as lethality of developing embryos. Both methods of exposure, waterborne and nanoinjection, resulted in similar dose-dependent effects. Some sublethal effects, such as edema at 48 hpf, delayed hatching, and curvature of the spine was observed after 72 hpf. In ovo, nanoinjection was deemed to be an accurate method of exposure for controlling the actual internal dose for study of adverse effects, which closely mimicked responses to waterborne exposure of zebrafish embryo to PFOS.

Possible role of serotonin and neuropeptide Y on the disruption of the reproductive axis activity by perfluorooctane sulfonate.
Lopez-Doval S., Salgado R., Fernandez-Perez B. and Lafuente A.
Toxicol Lett. 2015;233(2):138-47.

Perfluorooctane sulfonate (PFOS) is an endocrine disruptor, whose exposure can induce several alterations on the reproductive axis activity in males during adulthood. This study was undertaken to evaluate the possible role of serotonin and neuropeptide Y (NPY) on the disruption of the hypothalamic-pituitary-testicular (HPT) axis induced by PFOS in adult male rats. For that, adult male rats were orally treated with 0.5; 1.0; 3.0 and 6.0mg of PFOS/kg/day for 28 days. After PFOS exposure, serotonin concentration increased in the anterior and mediobasal hypothalamus as well as in the median eminence. The metabolism of this amine (expressed as the ratio 5-hydroxyindolacetic acid (5-HIAA)/serotonin) was diminished except in the anterior hypothalamus, with the doses of 3.0 and 6.0mg/kg/day, being this dose 0.5mg/kg/day in the median eminence. In general terms, PFOS-treated rats presented a decrease of the hypothalamic concentration of the gonadotropin releasing hormone (GnRH) and NPY. A diminution of the serum levels of the luteinizing hormone (LH), testosterone and estradiol were also shown. These results suggest that both serotonin and NPY could be involved in the inhibition induced by PFOS on the reproductive axis activity in adult male rats.

Elevated blood pressure in offspring of rats exposed to diverse chemicals during pregnancy.
Rogers J. M., Ellis-Hutchings R. G., Grey B. E., Zucker R. M., Norwood J., Jr., Grace C. E., Gordon C. J. and Lau C.
Toxicol Sci. 2014;137(2):436-46.

Adverse intrauterine environments have been associated with increased risk of later cardiovascular disease and hypertension. In an animal model using diverse developmental toxicants, we measured blood pressure (BP), renal nephron endowment, renal glucocorticoid receptor (GR) gene expression, and serum aldosterone in offspring of pregnant Sprague Dawley rats exposed to dexamethasone (Dex), perfluorooctane sulfonate (PFOS), atrazine, perfluorononanoic acid (PFNA), arsenic, or nicotine. BP was assessed by tail cuff photoplethysmography, nephron endowment by confocal microscopy, and renal GR mRNA by qPCR. BP was also measured by telemetry, and corticosterone (CORT) was measured in resting or restrained Dex and atrazine offspring. Treated dams gained less weight during treatment in all groups except arsenic. There were chemical- and sex-specific effects on birth weight, but offspring body weights were similar by weaning. BP was higher in Dex, PFOS, atrazine, and PFNA male offspring by 7-10 weeks. Female offspring exhibited elevated BP at 10 weeks for PFNA and arsenic, and at 37 weeks for Dex, PFOS, and atrazine. Dex, PFOS, and atrazine offspring still exhibited elevated BP at 52-65 weeks of age; others did not. Elevated BP was associated with lower nephron counts. Dex, PFOS, and atrazine offspring had elevated renal GR gene expression. Elevations in BP were also observed in Dex and atrazine offspring by radiotelemetry. Atrazine offspring exhibited enhanced CORT response to restraint. Elevated offspring BP was induced by maternal exposure to toxicants. Because all treatments affected maternal gestational weight gain, maternal stress may be a common underlying factor in these observations.

Perfluorooctane sulfonate effects on the reproductive axis in adult male rats.
Lopez-Doval S., Salgado R., Pereiro N., Moyano R. and Lafuente A.
Environ Res. 2014;134:158-68.

Perfluorooctane sulfonate (PFOS) is a neurotoxic agent and it can disrupt the endocrine system activity. This work was undertaken to evaluate the possible effects of PFOS exposure on the hypothalamic-pituitary-testicular axis (HPT) in adult male rats, and to evaluate the possible morphological alterations induced by PFOS in the endocrine tissues of this axis. Adult male rats were orally treated with 0.5; 1.0; 3.0 and 6.0 mg of PFOS/kg/day for 28 days. After PFOS exposure, hypothalamic noradrenaline concentration increased in the anterior hypothalamus and in the median eminence, not

changing in the mediobasal hypothalamus. PFOS treated rats presented a decrease of the gonadotropin releasing hormone (GnRH) gene expression, increasing the mRNA levels of the luteinizing hormone (LH) in rats treated with all doses administered except with the dose of 6 mg/kg/day. PFOS also induced a raise of the follicle stimulating hormone (FSH) gene expression in the animals exposed to 0.5 and 1.0 mg of PFOS/kg/day. After PFOS exposure, hypothalamic GnRH concentration was modified, LH and testosterone release was inhibited and FSH secretion was stimulated. Moreover, PFOS induced several histopathological alterations in the hypothalamus, pituitary gland and testis. The results obtained in the present study suggest in general terms that PFOS can inhibit the physiological activity of the reproductive axis in adult male rats, which could be explained, at least in part, by the structural alterations showed in the animals exposed to this chemical: very dense chromatin, condensed ribosomes and a loss of the morphology in the hypothalamus; a degeneration of the gonadotrophic cells, as well as a loss and degeneration of the spermatozoids and a very marked edema in the testis.

Early life perfluorooctanesulphonic acid (PFOS) exposure impairs zebrafish organogenesis.
Chen J., Tanguay R. L., Tal T. L., Gai Z., Ma X., Bai C., Tilton S. C., Jin D., Yang D., Huang C. and Dong Q.
Aquat Toxicol. 2014;150:124-32.

As a persistent organic contaminant, perfluorooctanesulphonic acid (PFOS) has been widely detected in the environment, wildlife, and humans. The present study revealed that zebrafish embryos exposed to 16 muM PFOS during a sensitive window of 48-96 hour post-fertilization (hpf) disrupted larval morphology at 120 hpf. Malformed zebrafish larvae were characterized by uninflated swim bladder, less developed gut, and curved spine. Histological and ultrastructural examination of PFOS-exposed larvae showed structural alterations in swim bladder and gut. Whole genome microarray was used to identify the early transcripts dysregulated following exposure to 16 muM PFOS at 96 hpf. In total, 1278 transcripts were significantly misexpressed ($p<0.05$) and 211 genes were changed at least two-fold upon PFOS exposure in comparison to the vehicle-exposed control group. A PFOS-induced network of perturbed transcripts relating to swim bladder and gut development revealed that misexpression of genes were involved in organogenesis. Taken together, early life stage exposure to PFOS perturbs various molecular pathways potentially resulting in observed defects in swim bladder and gut development.

Glucose and lipid homeostasis in adult rat is impaired by early-life exposure to perfluorooctane sulfonate.
Lv Z., Li G., Li Y., Ying C., Chen J., Chen T., Wei J., Lin Y., Jiang Y., Wang Y., Shu B., Xu B. and Xu S.
Environ Toxicol. 2013;28(9):532-42.

Perfluorooctane sulfonate (PFOS), which belongs to the degradation product of many perfluorinated compounds, is on the list of persistent organic pollutants (POPs) and is currently detected in both wildlife and humans. The consequence of gestational and lactational exposure to PFOS on prediabetes effect in offspring was investigated in rats in the present study. Maternal rats were treated with vehicle, 0.5 mg/kg/day or 1.5 mg/kg/day PFOS respectively from gestation day 0 to postnatal day 21. The glucose and lipid metabolism effects were investigated on the offspring in adulthood. The gestational and lactational exposure to PFOS led to low body weight from birth to weaning, and evoked signs of a prediabetic state, with elevated fasting serum insulin and leptin level, impaired glucose tolerance, though the fasting serum glucose and glycosylated serum protein level were normal. Abnormal lipid homeostasis was also observed by the phenomenon of hepatic steatosis and increased gonadal fat pad weight. However, the circulating serum level of fasting triglyceride and cholesterol level were no different from controls. Our results suggested that developmental exposure to PFOS may contribute to glucose and lipid metabolic disorder in adulthood.

Combined effects of PFOS and PFOA on zebrafish (Danio rerio) embryos.
Ding G., Zhang J., Chen Y., Wang L., Wang M., Xiong D. and Sun Y.
Arch Environ Contam Toxicol. 2013;64(4):668-75.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are two kinds of emerging contaminants most studied in recent years. However, there is limited information about their combined toxicity to aquatic organisms. In the present study, the single and combined toxicity of PFOA and PFOS to zebrafish (Danio rerio) embryos were investigated. PFOS was more toxic than PFOA for the single toxicity. In four mixtures, PFOS and PFOA showed complex interactive effects that changed from additive to synergistic effect, then to antagonistic effect, and at last turnover to synergic effect again, with increased molar ratios of PFOS. Neither the concentration-addition model nor the independent-action model could predict the combined effects when strong interactive effects existed. Although the interactive effects of PFOS and PFOA affected their combined toxicity, the trend of mixture toxicity still showed an increase with increasing molar ratios of PFOS in the mixture.

Prenatal PFOS exposure induces oxidative stress and apoptosis in the lung of rat off-spring.
Chen T., Zhang L., Yue J. Q., Lv Z. Q., Xia W., Wan Y. J., Li Y. Y. and Xu S. Q.
Reprod Toxicol. 2012;33(4):538-45.

Perfluorooctane sulfonate (PFOS) could induce neonatal pulmonary injuries in rodents. The aim of this study was to investigate the underlying mode of action. Pregnant rats were dosed orally with PFOS (0, 0.1 and 2.0mg/kgd) from gestation days (GD) 1 to 21. Lung samples from postnatal day (PND) 0 and 21 pups were analyzed for the toxic effects of PFOS. The results showed that maternal exposure to 2.0mg/kgd PFOS caused severe histopathological changes along with marked oxidative injuries and cell apoptosis in offspring lungs; at the same time, the ratio of Bax to Bcl-2, release of cytochrome c (Cyt c) from mitochondria to cytoplasm, expressions of Fas and Fas-L, and activities of caspase-3, -8 and -9 were up-regulated correspondingly. The results indicate that oxidative stress and both intrinsic and extrinsic cell death pathways were involved in prenatal PFOS exposure-induced injuries in postnatal lungs.

Prenatal exposure to perfluorooctanesulfonate in rat resulted in long-lasting changes of expression of synapsins and synaptophysin.
Zeng H. C., Li Y. Y., Zhang L., Wang Y. J., Chen J., Xia W., Lin Y., Wei J., Lv Z. Q., Li M. and Xu S. Q.
Synapse. 2011;65(3):225-33.

Both animal and human studies have demonstrated that exposure to chemical pollutants during critical developmental period causes adverse consequences later in life. In uterus, perfluorooctanesulfonate (PFOS) exposure has been known to cause developmental neurotoxicity, such as increased motor activity, reduced habitation and impaired cognitive function. The possible mechanism of the impaired cognitive function induced by prenatal PFOS exposure was evaluated in this study. Pregnant Sprague Dawley (SD) rats were given 0.1, 0.6, and 2.0 mg kg(-1) birth weight (bw) d(-1) by gavage from gestation day (GD) 0 to GD20. Control received 0.5% Tween-20 vehicle (4 ml kg(-1) bw d(-1)). PFOS concentration in hippocampus of offspring was observed on postnatal day (PND) 0 and PND21. The ultrastructure of hippocampus and the gene expression of synaptic vesicle associated proteins in offspring hippocampus, which were important for the neurotransmitter release, were investigated. The transmission electron photomicrographs of the offspring hippocampus from PFOS-treated maternal groups showed the ultrastructure of synapses was negatively affected. The offspring from PFOS-treated maternal groups also differed significantly from controls with respect to the expression of synaptic vesicle associated proteins. The mRNA levels of

synapsin1 (Syn1), synapsin2 (Syn2), and synaptophysin (Syp) were decreased in treated groups either on PND0 or on PND21. However, the mRNA level of synapsin3 (Syn3) decreased in 0.6- and 2.0-mg kg(-1) group on PND0, and showed no significant difference among control group and all treated groups on PND21. These results indicate that the impairment of cognitive function induced by PFOS may be attributed to the lower mRNA levels of synaptic vesicle associated proteins and the change of synaptic ultrastructure in hippocampus.

PFOS prenatal exposure induce mitochondrial injury and gene expression change in hearts of weaned SD rats.
Xia W., Wan Y., Li Y. Y., Zeng H., Lv Z., Li G., Wei Z. and Xu S. Q.
Toxicology. 2011;282(1-2):23-9.

Xenobiotics exposure in early life may have adverse effects on animals' development through mitochondrial injury or dysfunction. The current study demonstrated the possibility of cardiac mitochondrial injury in prenatal PFOS-exposed weaned rat heart. Pregnant Sprague-Dawley (SD) rats were exposed to perfluorooctane sulfonate (PFOS) at doses of 0.1, 0.6 and 2.0 mg/kg/d and 0.05% Tween 80 as control by gavage from gestation days 2-21. The dams were allowed to give nature delivery and then heart tissues from weaned (postnatal day 21) offspring rats were analyzed for mitochondrial injury through ultrastructure observation by electron microscope, global gene expression profile by microarray, as well as related mRNA and proteins expression levels by quantitative PCR and western blot. Ultrastructural analysis revealed significant vacuolization and inner membrane injury occurred at the mitochondria of heart tissues from 2.0 mg/kg/d dosage group. Meanwhile, the global gene expression profile showed significant difference in level of some mRNA expression associated with mitochondrial function at 2.0 mg/kg/d dosage group, compared to the control. Furthermore, dose-response trends for the expression of selected genes were analyzed by quantitative PCR and western blot analysis. The selected genes were mainly focused on those encoding for proteins involved in energy production, control of ion levels, and maintenance of heart function. The down-regulation of mitochondrial ATP synthetase (ATP5E, ATP5I and ATP5O) implicated a decrease in energy supply. This was accompanied by down-regulation of gene transcripts involved in energy consumption such as ion transporting ATPase (ATP1A3 and ATP2B2) and inner membrane protein synthesis (SLC25A3, SLC25A4, SLC25A10, SLC25A29). The up-regulation of gene transcripts encoding for uncoupling proteins (UCP1 and UCP3), epidermal growth factor receptor (EGFR) and connective tissue growth factor (CTGF), was probably a protective process to maintain heart function. The results indicate PFOS prenatal exposure can

induce cardiac mitochondrial injury and gene transcript change, which may be a significant mechanism of the developmental toxicity of PFOS to rat.

Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner.
Onishchenko N., Fischer C., Wan Ibrahim W. N., Negri S., Spulber S., Cottica D. and Ceccatelli S.
Neurotox Res. 2011;19(3):452-61.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are organic surfactants widely used in various industrial and consumer applications. Due to their chemical properties, these perfluorinated compounds (PFCs) have also become persistent contaminants. The risk of possible intrauterine and lactational exposure to these chemicals poses a significant health concern for potential developmental effects. In the present study we have found that dietary exposure of mice to 0.3 mg/kg of PFOS or PFOA throughout pregnancy results in different distribution pattern in the offspring brain and liver. In particular, exposure to PFOS led to four times higher accumulation of the chemical in the brains of newborn mice than PFOA. We have used a battery of behavioral tests to evaluate motor function, circadian activity, and emotion-related behavior in the exposed offspring. Exposure to PFOS resulted in decreased locomotion in a novel environment and reduced muscle strength only in male offspring. Prenatal exposure to PFOA was associated with changes in exploratory behavior in male and female offspring, as well as with increased global activity in males in their home cage. The neurobehavioral outcome of prenatal exposure to PFCs in mice is characterized by mild alterations in motor function and it appears to be sex-related.

Prenatal exposure to PFOS caused mitochondia-mediated apoptosis in heart of weaned rat.
Zeng H. C., He Q. Z., Li Y. Y., Wu C. Q., Wu Y. M. and Xu S. Q.
Environ Toxicol. 2014.

Perfluorooctanyl sulfonate (PFOS), a cardiac toxicity compound, has been widely detected in the environment and in organisms. However, the toxic mechanism is not clear. Our previous study indicated that prenatal PFOS exposure led to swollen mitochondrial with vacuolar structure and loss of cristae in offsping's heart. The purpose of this study was to investigate the effect of PFOS on the apoptosis in developing heart and mitochondria-mediated apoptosis pathway. Pregnant Sprague-Dawley (SD) rats were exposed to PFOS at doses of 0.1, 0.6, and 2.0 mg/kg-d and 0.05% Tween 80 as control by gavage from gestation day 2 (GD 2) to GD 21. Apoptosis, as well as

expression of apoptosis related genes associated with mitochondrial-mediated apoptosis pathway, including p53, bcl-2, bax, cytochrome c, caspase-9, and caspase-3 were analyzed in heart tissues from weaned (postnatal day 21, PND 21) offspring. The results showed that prenatal PFOS exposure resulted in apoptosis in the offspring's heart. The mRNA and protein expression levels of p53, bax, cytochrome c, caspase-9, and caspase-3 in the offspring's heart were enhanced in various PFOS-treated groups, meanwhile, the bcl-2 expression levels were decreased. Our results indicated that prenatal PFOS exposure induced the apoptosis of weaned offspring rat heart tissue via mitochondria-mediated apoptotic pathway.

Combined effects of perfluorooctane sulfonate (PFOS) and maternal restraint stress on hypothalamus adrenal axis (HPA) function in the offspring of mice.
Ribes D., Fuentes S., Torrente M., Colomina M. T. and Domingo J. L.
Toxicol Appl Pharmacol. 2010;243(1):13-8.

Although it is known that prenatal exposure to perfluorooctane sulfonate (PFOS) can cause developmental adverse effects in mammals, the disruptive effects of this compound on hormonal systems are still controversial. Information concerning the effects of PFOS on hypothalamus adrenal (HPA) axis response to stress and corticosterone levels is not currently available. On the other hand, it is well established that stress can enhance the developmental toxicity of some chemicals. In the present study, we assessed the combined effects of maternal restraint stress and PFOS on HPA axis function in the offspring of mice. Twenty plug-positive female mice were divided in two groups. Animals were given by gavage 0 and 6 mg PFOS/kg/day on gestation days 12-18. One half of the animals in each group were also subjected to restraint stress (30 min/session, 3 sessions/day) during the same period. Five plug-positive females were also included as non-manipulated controls. At 3 months of age, activity in an open-field and the stress response were evaluated in male and female mice by exposing them to 30 min of restraint stress. Male and female offspring were subsequently sacrificed and blood samples were collected to measure changes in corticosterone levels at four different moments related to stress exposure conditions: before stress exposure, immediately after 30 min of stress exposure, and recuperation levels at 60 and 90 min after stress exposure. Results indicate corticosterone levels were lower in mice prenatally exposed to restraint. In general terms, PFOS exposure decreased corticosterone levels, although this effect was only significant in females. The recuperation pattern of corticosterone was mainly affected by prenatal stress. Interactive effects between PFOS and maternal stress were sex dependent. The current results suggest that prenatal PFOS exposure induced long-lasting effects in mice.

Yu W. G., Liu W., Jin Y. H., Liu X. H., Wang F. Q., Liu L. and Nakayama S. F.

Environ Sci Technol. 2009;43(21):8416-22.

Perfluorooctane sulfonate (PFOS), an environmentally persistent organic pollutant, has been reported to be transferred to the developing organisms via both placenta and breast milk. A cross-foster model was used to determine whether prenatal or postnatal exposure to PFOS alone can disturb the TH homeostasis in rat pups, and if so, which kind of exposure is a major cause of TH level alteration. Pregnant rats were fed standard laboratory rodent diet containing 0 (control) or 3.2 mg PFOS/kg throughout gestation and lactation period. On the day of birth, litters born to treated and control dams were cross-fostered, resulting in the following groups: unexposed control (CC), pups exposed only prenatally (TC), only postnatally (CT) or both prenatally and postnatally (TT). Serum and liver PFOS concentrations, serum total thyroxine (T4), total triiodothyronine (T3), reverse T3 (rT3) levels, and hepatic expression of genes involved in TH transport, metabolism, and receptors were evaluated in pups at the age of postnatal days (PNDs) 0, 7, 14, 21, or 35. PFOS body burden level in pups in group CT increased, while those in group TC dropped as they aged. Neither total T3 nor rT3 in pups was affected by PFOS exposure. Gestational exposure to PFOS alone (TC) significantly ($p < 0.05$) decreased T4 level in pups on PNDs 21 and 35, 20.3 and 19.4% lower than the control on the same PND, respectively. Postnatal exposure to PFOS alone (CT) also induced T4 depression on PNDs 21 and 35, 28.6 and 35.9% lower than controls, respectively. No significant difference in T4 level ($p > 0.05$) was observed between TC and CT on these two time points. None of the selected TH related transcripts was affected by PFOS in pups on PND 0. Only transcript level of transthyretin, TH binding protein, in group TT significantly increased to 150% of the control on PND 21. The results showed that prenatal PFOS exposure and postnatal PFOS exposure induced hypothyroxinemia in rat pups to a similar extent, which suggested that in utero PFOS exposure and postnatal PFOS accumulation, especially though maternal milk, are matters of great concern.

[Effects of perfluorooctane sulfonate on learning and memory of rat pups].
Liu L., Jin Y. H., Wang L., Yu H. Y., Liu W., Yu Q. L., Wang K., Liu B. and Wang J.
Zhonghua Yu Fang Yi Xue Za Zhi. 2009;43(7):622-7.

OBJECTIVE: To study the effects of prenatal and postnatal perfluorooctane sulfonate (PFOS) exposure on spatial learning and memory, N-methyl-D-aspartate receptor 2B (NR2B) mRNA and protein level in frontal cortex and hippocampus of rat pups and to explore the mechanism of developmental neurotoxicity induced by PFOS. METHODS:

Twenty-eight pregnant rats were randomly divided into three groups in proportion of 3:2:2, including control group (C), low dose group (L) and high dose group (H) by means of randomized number table, which respectively received 0, 7.2, 14.4 mg/kg PFOS feed from pregnancy day 0 to postnatal day (PND) 30 by free feedings. The animal models of prenatal and postnatal non-exposure (CC), prenatal exposure (LC and HC), postnatal exposure (CL and CH), and prenatal and postnatal exposure (LL and HH) to PFOS were established by cross-fostering method. The spatial learning and memory were measured by water maze experiment,the NR2B mRNA levels in frontal cortex of rat pups was determined with semi-quantitative RT-PCR, NR2B protein express in cerebral cortex (frontal and temporal cortex) and hippocampus (CA1, CA3, CA4 and DG regions) of rat pups was detected by immunohistochemistry. RESULTS: The escape latency of CL, CH, LL and HH groups pups in water maze experiment were (99.83 +/- 25.77) s, (111.30 +/- 17.82) s, (106.40 +/- 18.71) s, (107.70 +/- 16.85) s, and longer as compared with CC group [(54.90 +/- 26.69) s] (q value were 4.349, 4.773, 6.026 and 5.641, respectively, P <0.01). The number of errors of HH group rat pups entering dead end was (22.30 +/- 7.56) at the training day 4, and it was significantly higher than that of CC group (9.80 +/- 4.64) (q = 5.173, P < 0.01). The NR2B mRNA levels of frontal cortex of pups in HC group at PND1, and LC group, HC group and HH group at PND14 were (0.167 +/- 0.008), (0.364 +/- 0.035), (0.341 +/- 0.030) and (0.328 +/- 0.045) respectively,which were significantly lower than CC group (0.271 +/- 0.060) and (0.465 +/- 0.067) (q values were 3.547, 3.739, 4.597 and 5.006, respectively, P< 0.05). The results of immunohistochemistry indicated that NR2B protein express of the hippocampus CA1 region of pups in LC group was (0.091 +/- 0.005), and showed significant lower than CC group which was (0.123 +/- 0.009) at PND1 (q = 5.209, P <0.05). At PND14, the effect of PFOS extended to cerebral cortex and hippocampus regions. At PND28, the effects of PFOS were showed in hippocampus CA1, CA3 and temporal cortex regions. CONCLUSION: Prenatal and postnatal exposure to PFOS should result in the spatial learning and memory damage,and the mechanism might be possibly involved in the decrease of NR2B level in cerebral cortex and hippocampal formation regions.

Developmental toxicity of perfluorooctane sulfonate (PFOS) is not dependent on expression of peroxisome proliferator activated receptor-alpha (PPAR alpha) in the mouse.
Abbott B. D., Wolf C. J., Das K. P., Zehr R. D., Schmid J. E., Lindstrom A. B., Strynar M. J. and Lau C.
Reprod Toxicol. 2009;27(3-4):258-65.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are members of a family of perfluorinated compounds. Both are environmentally persistent and found in the serum of wildlife and humans. PFOS and PFOA are developmentally toxic in laboratory rodents. Exposure to these chemicals in utero delays development and reduces postnatal survival and growth. Exposure to PFOS on the last 4 days of gestation in the rat is sufficient to reduce neonatal survival. PFOS and PFOA are weak agonists of peroxisome proliferator activated receptor-alpha (PPAR alpha). The reduced postnatal survival of neonatal mice exposed to PFOA was recently shown to depend on expression of PPAR alpha. This study used PPAR alpha knockout (KO) and 129S1/SvlmJ wild type (WT) mice to determine if PPAR alpha expression is required for the developmental toxicity of PFOS. After mating overnight, the next day was designated gestation day (GD) 0. WT females were weighed and dosed orally from GD15 to 18 with 0.5% Tween-20, 4.5, 6.5, 8.5, or 10.5mg PFOS/kg/day. KO females were dosed with 0.5% Tween-20, 8.5 or 10.5mg PFOS/kg/day. Dams and pups were observed daily and pups were weighed on postnatal day (PND) 1 and PND15. Eye opening was recorded from PND12 to 15. Dams and pups were killed on PND15, body and liver weights recorded, and serum collected. PFOS did not affect maternal weight gain or body or liver weights of the dams on PND15. Neonatal survival (PND1-15) was significantly reduced by PFOS in both WT and KO litters at all doses. WT and KO pup birth weight and weight gain from PND1 to 15 were not significantly affected by PFOS exposure. Relative liver weight of WT and KO pups was significantly increased by the 10.5mg/kg dose. Eye opening of PFOS-exposed pups was slightly delayed in WT and KO on PND13 or 14, respectively. Because results in WT and KO were comparable, it is concluded that PFOS-induced neonatal lethality and delayed eye opening are not dependent on activation of PPAR alpha.

Neonatal death of mice treated with perfluorooctane sulfonate.
Yahia D., Tsukuba C., Yoshida M., Sato I. and Tsuda S.
J Toxicol Sci. 2008;33(2):219-26.

Pregnant mice exposure to perfluorooctane sulfonate (PFOS) causes neonatal death. Ten pregnant ICR mice per group were given 1, 10 or 20 mg/kg PFOS daily by gavage

from gestational day (GD) 0 to the end of the study. Five dams per group were sacrificed on GD 18 for prenatal evaluation, the others were left to give birth. Additional studies were conducted for histopathological examination of lungs and heads of fetuses and neonates at birth. PFOS treatment (20 mg/kg) reduced the maternal weight gain and feed intake but increased the water intake. The liver weight increased in a dose-dependent manner accompanied by hepatic hypertrophy at 20 mg/kg. PFOS reduced the fetal body weight in a dose-dependent manner and caused a bilateral enlargement in the neck region in all fetuses at 20 mg/kg and mild enlargement in some fetuses at 10 mg/kg, in addition to skeletal malformations. Almost all fetuses at 20 mg/kg were alive on GD18 and showed normal lung structure; but at parturition, all neonates were inactive and weak, showed severe lung atelectasis and severe dilatation of intracranial blood vessel, and died within a few hours. At 10 mg/kg, all neonates were born alive, 27% showed slight lung atelectasis, all of them had mild to severe dilatation of the intracranial blood vessel, and 45% of neonates died within 24 hr. The cause of neonatal death in mice exposed to PFOS may be attributed either to the intracranial blood vessel dilatation or to respiratory dysfunction. The former might be a cause of the latter.

Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice.
Keil D. E., Mehlmann T., Butterworth L. and Peden-Adams M. M.
Toxicol Sci. 2008;103(1):77-85.

Perfluorinated alkyl acids (PFAAs) are used in a multitude of applications and are categorized as high-production volume chemicals produced in quantities exceeding 10,000 lbs/year. As a result, widespread exposure has been documented in adults, children, and infants. It is generally accepted that children are more sensitive to the effects of xenobiotic exposures during fetal and postnatal periods of development; therefore, considerable efforts are required to investigate the potential impact of a model PFAA, perfluorooctane sulfonate (PFOS) on children's immunological health. Using the pairing of female C57BL/6N mice with male C3H/HeJ, developmental immunotoxicity was evaluated in B6C3F1 pups following oral maternal exposure to PFOS on gestations days 1-17. Exposure levels included 0.1, 1, and 5 mg/kg/day PFOS. Natural killer (NK) cell activity, SRBC IgM plaque assay, CD4/8 lymphocytic subpopulations, nitrite production in peritoneal macrophages, and body/organ weights were evaluated at 4 and 8 weeks of age in F1 pups. No significant dose-responsive changes in maternal or pup body weights, flow cytometry, or macrophage function were observed, yet hepatomegaly was indicated in F1 male pups at 4 weeks of age. Functional deficits were not evident until 8 weeks of age when NK cell function and IgM production were significantly decreased. When compared with females, male pups were

more sensitive to the effects of PFOS thereby establishing a no observed adverse effect level and low observed adverse effect level of 0.1 and 1.0 mg/kg/day (males only) following maternal PFOS exposure level, respectively. This study establishes that the developing immune system is sensitive to the effects of PFOS and results in functional deficits in innate and humoral immunity detectable at adulthood.

Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behavior of the offspring.
Fuentes S., Colomina M. T., Vicens P., Franco-Pons N. and Domingo J. L.
Toxicol Sci. 2007;98(2):589-98.

The combined effects of maternal restraint stress and perfluorooctane sulfonate (PFOS) on postnatal development and behavior of the offspring were assessed in mice. Thirty-four plug positive females were randomly divided into two groups. Animals were given by gavage 0 and 6 mg PFOS/kg/day on gestation days 12-18. One-half of the animals in each group was subjected to restraint stress (30 min per session, three sessions per day) during the same period. Neither restraint nor PFOS exposure significantly modified maternal food or water consumption. Pups of dams exposed to 6 mg/kg of PFOS showed a reduced body weight on postnatal days 4 and 8. Moreover, PFOS exposure induced some delay in developmental landmarks and neuromotor maturation. Maternal restraint stress reduced activity in an open-field when combined with 6 mg PFOS/kg/day. In addition, in males prenatal restraint stress impaired motor coordination in a rotarod. The current results indicate that concurrent exposure to PFOS and restraint stress during pregnancy induces opposite effects on developmental parameters in the pups. These effects consist in a general delayed maturation trend induced by PFOS exposure, and a general accelerated maturation pattern induced by prenatal stress. Interactive effects between PFOS and maternal stress were observed in young adult mice. These effects consisted mainly in a diminished activity in an open-field test.

Influence of maternal restraint stress on the long-lasting effects induced by prenatal exposure to perfluorooctane sulfonate (PFOS) in mice.
Fuentes S., Colomina M. T., Vicens P. and Domingo J. L.
Toxicol Lett. 2007;171(3):162-70.

The behavioral effects of concurrent maternal exposure to restraint stress and perfluorooctane sulfonate (PFOS) were assessed in the offspring of mice at 3 months of age. Plug positive females were divided into two groups. Animals were given by gavage 0 and 6mg PFOS/kg/day on gestation days 12-18. One-half of the animals in each

group were subjected to restraint stress (30min/session, three sessions per day) during the same period. At 3 months, mice were evaluated for general activity in an open-field, and for learning and memory in a water maze task. The group prenatally exposed to PFOS and restraint presented a reduced mobility in the open-field. In the water maze, an interaction between sex and restraint was observed. Delayed task learning was also detected in females prenatally exposed to PFOS and restraint. An overall effect of restraint was observed in mice on retention of the task, suggesting a better retention in restrained animals. On the other hand, corticosterone levels were lower in animals prenatally subjected to restraint stress. The current results suggest interactive effects between PFOS and maternal stress.

Interactions in developmental toxicology: concurrent exposure to perfluorooctane sulfonate (PFOS) and stress in pregnant mice.
Fuentes S., Colomina M. T., Rodriguez J., Vicens P. and Domingo J. L.
Toxicol Lett. 2006;164(1):81-9.

The maternal and developmental toxicity of combined exposure to restraint stress and perfluorooctane sulfonate (PFOS) was assessed in mice. On gestation Days 6-18, four groups of plug-positive female mice were orally exposed to PFOS at 0, 1.5, 3 and 6 mg/kg/day. Four additional groups of plug-positive animals received the same PFOS doses being restrained during 30 min three times per day. A control group was also included. Cesarean sections were performed on Day 18 of gestation and fetuses were weighed and examined for external, internal and skeletal malformations and variations. Before sacrifice of the dams, blood was collected and serum samples were prepared for thyroid hormones (total and free T3 and T4) and corticosterone analyses. The results of the present study show that both PFOS and restraint stress induced maternal toxicity. In turn, PFOS-induced fetal toxicity was evidenced by increased prenatal mortality. The only effect of restraint on fetal toxicity was a reduction on body weight and an increased prenatal mortality in fetuses concurrently exposed to 1.5 mg/kg of PFOS and restraint. PFOS-induced adverse effects on maternal and fetal toxicity in mice were observed at lower doses than those previously reported.

Effects of prenatal perfluorooctane sulfonate (PFOS) exposure on lung maturation in the perinatal rat.
Grasty R. C., Bjork J. A., Wallace K. B., Wolf D. C., Lau C. S. and Rogers J. M.
Birth Defects Res B Dev Reprod Toxicol. 2005;74(5):405-16.

BACKGROUND: Perfluorooctane sulfonate (PFOS), found widely in wildlife and humans, is environmentally and metabolically stable. Environmental PFOS may be from

its use as a surfactant, hydrolysis of perfluorooctanesulfonyl fluoride, and degradation of N-alkyl-perfluorooctanesulfonamide compounds formerly used in numerous applications. Prenatal exposure to PFOS in rodents causes neonatal mortality; treatment on gestation days (GD) 19-20 is sufficient to induce neonatal death in rats. Affected pups are born alive but present with labored breathing. Their lungs are pale and often do not expand fully on perfusion. METHODS: Pregnant Sprague-Dawley rats received 0, 25, or 50 mg/kg/day PFOS/K+ orally on GD 19-20. Lungs from GD 21 fetuses and neonates were prepared for histology and morphometry. Rescue experiments included co-administration of dexamethasone or retinyl palmitate with PFOS. Pulmonary surfactant was investigated with mass spectrometry in GD 21 amniotic fluid and neonatal lungs. Microarray analysis was carried out on PND 0 lungs. RESULTS: Histologically, alveolar walls were thicker in lungs of PFOS-exposed newborns compared to controls. The ratio of solid tissue:small airway was increased, suggesting immaturity. Rescue studies were ineffective. Phospholipid concentrations and molecular speciation were unaffected by PFOS. No changes in markers of alveolar differentiation were detected by microarray analysis. CONCLUSIONS: Morphometric changes in lungs of PFOS exposed neonates were suggestive of immaturity, but the failure of rescue agents and normal pulmonary surfactant profile indicate that the labored respiration and mortality observed in PFOS-treated neonates was not due to lung immaturity.

Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters.
Luebker D. J., York R. G., Hansen K. J., Moore J. A. and Butenhoff J. L.
Toxicology. 2005;215(1-2):149-69.

Perfluorooctanesulfonate (PFOS) is a widely distributed, environmentally persistent acid found at low levels in human, wildlife, and environmental media samples. Neonatal mortality has been observed following PFOS exposure in a two-generation reproduction study in rats and after dosing pregnant rats and mice during gestation. Objectives of the current study were to better define the dose-response curve for neonatal mortality in rat pups born to PFOS-exposed dams and to investigate biochemical and pharmacokinetic parameters potentially related to the etiology of effects observed in neonatal rat pups. In the current study, additional doses of 0.8, 1.0, 1.2, and 2.0 mg/kg/day were included with original doses used in the two-generation study of 0.4 and 1.6 mg/kg/day in order to obtain data in the critical range of the dose-response curve. Biochemical parameters investigated in dams and litters included: (1) serum lipids, glucose, mevalonic acid, and thyroid hormones; (2) milk cholesterol; and (3) liver lipids. Pharmacokinetic parameters

investigated included the interrelationship of administered oral dose of PFOS to maternal body burden of PFOS and the transfer of maternal body burden to the fetus in utero and pup during lactation, as these factors may affect neonatal toxicity. Dosing of dams occurred for 6 weeks prior to mating with untreated breeder males, through confirmed mating, gestation, and day four of lactation. Dose levels for the dose-response and etiological investigation were 0.0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg/day PFOS. Statistically significant decreases in gestation length were observed in the 0.8 mg/kg and higher dose groups. Decreases in viability through lactation day 5 were observed in the 0.8 mg/kg and higher dose groups, becoming statistically significant in the 1.6 and 2.0 mg/kg dose groups. Reduced neonatal survival did not appear to be the result of reductions in lipids, glucose utilization, or thyroid hormones. The endpoints of gestation length and decreased viability were positively correlated, suggesting that late-stage fetal development may be affected in pups exposed to PFOS in utero and may contribute to the observed mortality. Benchmark dose (BMD) estimates for decreased gestation length, birth weight, pup weight on lactation day 5, pup weight gain through lactation day 5, and viability resulted in values ranging from 0.27 to 0.89mg/kg/day for the lower 95% confidence limit of the BMD5 (BMDL5). Results of analyses for PFOS in biological matrices indicate a linear proportionality of mean serum PFOS concentration to maternal administered dose prior to mating and through the first two trimesters of gestation. However, at 21 days of gestation, mean serum PFOS concentrations were notably reduced from values measured earlier in gestation. Urinary and fecal elimination was low as expected from prior observations in adult rats. Significant transfer of PFOS from dam to fetus in utero was confirmed, and results suggest that dam and corresponding fetal body burdens, as indicated by serum and liver PFOS levels, correlate with neonatal survival.

Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats.
Luebker D. J., Case M. T., York R. G., Moore J. A., Hansen K. J. and Butenhoff J. L. Toxicology. 2005;215(1-2):126-48.

Perfluorooctanesulfonate (PFOS) is a persistent acid found widely distributed in wildlife and humans. To understand the potential reproductive and developmental effects of PFOS, a two-generation reproduction study was conducted in rats. Male and female rats were dosed via oral gavage at dose levels of 0, 0.1, 0.4, 1.6, and 3.2 mg/(kg day) for 6 weeks prior to mating, during mating, and, for females, through gestation and lactation, across two generations. Due to substantial F1 neonatal toxicity observed in the 1.6 and 3.2 mg/(kg day) groups, continuation into the second generation was limited to F1 pups from the 0, 0.1, and 0.4 mg/(kg day) groups. No adverse effects were

observed in F0 females or their fetuses upon caesarean sectioning at gestation day 10. Statistically significant reductions in body-weight gain and feed consumption were observed in F0 generation males and females at dose levels of 0.4 mg/(kg day) and higher, but not in F1 adults. PFOS did not affect reproductive performance (mating, estrous cycling, and fertility); however, reproductive outcome, as demonstrated by decreased length of gestation, number of implantation sites, and increased numbers of dams with stillborn pups or with all pups dying on lactation days 1-4, was affected at 3.2 mg/(kg day) in F0 dams. These effects were not observed in F1 dams at the highest dose tested, 0.4 mg/(kg day). Neonatal toxicity in F1 pups, as demonstrated by reduced survival and body-weight gain through the end of lactation, occurred at a maternal dose of 1.6 mg/(kg day) and higher while not at dose levels of 0.1 or 0.4 mg/(kg day) or in F2 pups at the 0.1 or 0.4 mg/(kg day) dose levels tested. In addition to these adverse effects, slight yet statistically significant developmental delays occurred at 0.4 (eye opening) and 1.6 mg/(kg day) (eye opening, air righting, surface righting, and pinna unfolding) in F1 pups. Based on these data, the NOAELs were as follows: reproductive function: F0 > or =3.2 and F1 > or =0.4 mg/(kg day); reproductive outcome: F0=1.6 and F1 > or =0.4 mg/(kg day); overall parental effects: F0=0.1 and F1 > or =0.4 mg/(kg day); offspring effects: F0=0.4 and F1 > or =0.4 mg/(kg day). To distinguish between maternal and pup influences contributing to the perinatal mortality observed in the two-generation study, a follow-up cross-foster study was performed. Results of this study indicated that in utero exposure to PFOS causally contributed to post-natal pup mortality, and that pre-natal and post-natal exposure to PFOS was additive with respect to the toxic effects observed in pups.

Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: maternal and prenatal evaluations.
Thibodeaux J. R., Hanson R. G., Rogers J. M., Grey B. E., Barbee B. D., Richards J. H., Butenhoff J. L., Stevenson L. A. and Lau C.
Toxicol Sci. 2003;74(2):369-81.

The maternal and developmental toxicities of perfluorooctane sulfonate (PFOS, C8F17SO3-) were evaluated in the rat and mouse. PFOS is an environmentally persistent compound used as a surfactant and occurs as a degradation product of both perfluorooctane sulfonyl fluoride and substituted perfluorooctane sulfonamido components found in many commercial and consumer applications. Pregnant Sprague-Dawley rats were given 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from gestational day (GD) 2 to GD 20; CD-1 mice were similarly treated with 1, 5, 10, 15, and 20 mg/kg PFOS from GD 1 to GD 17. Controls received 0.5% Tween-20 vehicle (1 ml/kg for rats and 10 ml/kg for mice). Maternal weight gain, food and water consumption, and serum

chemistry were monitored. Rats were euthanized on GD 21 and mice on GD 18. PFOS levels in maternal serum and in maternal and fetal livers were determined. Maternal weight gains in both species were suppressed by PFOS in a dose-dependent manner, likely attributed to reduced food and water intake. Serum PFOS levels increased with dosage, and liver levels were approximately fourfold higher than serum. Serum thyroxine (T4) and triiodothyronine (T3) in the PFOS-treated rat dams were significantly reduced as early as one week after chemical exposure, although no feedback response of thyroid-stimulating hormone (TSH) was observed. A similar pattern of reduction in T4 was also seen in the pregnant mice. Maternal serum triglycerides were significantly reduced, particularly in the high-dose groups, although cholesterol levels were not affected. In the mouse dams, PFOS produced a marked enlargement of the liver at 10 mg/kg and higher dosages. In the rat fetuses, PFOS was detected in the liver but at levels nearly half of those in the maternal counterparts, regardless of administered doses. In both rodent species, PFOS did not alter the numbers of implantations or live fetuses at term, although small deficits in fetal weight were noted in the rat. A host of birth defects, including cleft palate, anasarca, ventricular septal defect, and enlargement of the right atrium, were seen in both rats and mice, primarily in the 10 and 20 mg/kg dosage groups, respectively. Our results demonstrate both maternal and developmental toxicity of PFOS in the rat and mouse.

Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation.
Lau C., Thibodeaux J. R., Hanson R. G., Rogers J. M., Grey B. E., Stanton M. E., Butenhoff J. L. and Stevenson L. A.
Toxicol Sci. 2003;74(2):382-92.

The postnatal effects of in utero exposure to perfluorooctane sulfonate (PFOS, C8F17SO3-) were evaluated in the rat and mouse. Pregnant Sprague-Dawley rats were given 1, 2, 3, 5, or 10 mg/kg PFOS daily by gavage from gestation day (GD) 2 to GD 21; pregnant CD-1 mice were treated with 1, 5, 10, 15, and 20 mg/kg PFOS from GD 1 to GD 18. Controls received 0.5% Tween-20 vehicle (1 ml/kg for rats and 10 ml/kg for mice). At parturition, newborns were observed for clinical signs and survival. All animals were born alive and initially appeared to be active. In the highest dosage groups (10 mg/kg for rat and 20 mg/kg for mouse), the neonates became pale, inactive, and moribund within 30-60 min, and all died soon afterward. In the 5 mg/kg (rat) and 15 mg/kg (mouse) dosage groups, the neonates also became moribund but survived for a longer period of time (8-12 h). Over 95% of these animals died within 24 h. Approximately 50% of offspring died at 3 mg/kg for rat and 10 mg/kg for mouse. Cross-fostering the PFOS-exposed rat neonates (5 mg/kg) to control nursing dams failed to

improve survival. Serum concentrations of PFOS in newborn rats mirrored the maternal administered dosage and were similar to those in the maternal circulation at GD 21; PFOS levels in the surviving neonates declined in the ensuing days. Small but significant and persistent growth lags were detected in surviving rat and mouse pups exposed to PFOS prenatally, and slight delays in eye opening were noted. Significant increases in liver weight were observed in the PFOS-exposed mouse pups. Serum thyroxine levels were suppressed in the PFOS-treated rat pups, although triiodothyronine and thyroid-stimulating hormone [TSH] levels were not altered. Choline acetyltransferase activity (an enzyme that is sensitive to thyroid status) in the prefrontal cortex of rat pups exposed to PFOS prenatally was slightly reduced, but activity in the hippocampus was not affected. Development of learning, determined by T-maze delayed alternation in weanling rats, was not affected by PFOS exposure. These results indicate that in utero exposure to PFOS severely compromised postnatal survival of neonatal rats and mice, and caused delays in growth and development that were accompanied by hypothyroxinemia in the surviving rat pups.

Prenatal window of susceptibility to perfluorooctane sulfonate-induced neonatal mortality in the Sprague-Dawley rat.
Grasty R. C., Wolf D. C., Grey B. E., Lau C. S. and Rogers J. M.
Birth Defects Res B Dev Reprod Toxicol. 2003;68(6):465-71.

The critical period for increased neonatal mortality induced by perfluorooctane sulfonate (PFOS) exposure was evaluated in the rat. Timed-pregnant Sprague-Dawley rats were treated by oral gavage with 25 mg/kg/d PFOS/K(+) on four consecutive days (gestation days (GD) 2-5, 6-9, 10-13, 14-17, or 17-20) or with 0, 25, or 50 mg/kg/d PFOS/K(+) on GD 19-20. Controls received vehicle (10 ml/kg 0.5% Tween-20) on these days. Maternal weight gain was reduced in treated animals during dosing, as were food and water consumption. Following a 4-day treatment, litter size at birth was unaffected while pup weight was similarly reduced in the three earliest PFOS groups. All PFOS groups experienced decreases in survival while controls remained near 100%. Neonatal survival decreased in groups dosed later during gestation, approaching 100% with dosing on GD 17-20. Most deaths occurred before postnatal day (PND) 4, with the majority in the first 24 hours. Maternal serum PFOS levels on GD 21 were higher in groups exhibiting higher mortality. Following a 2-day treatment, PFOS groups experienced significant pup mortality by PND 1. Neonatal mortality continued through PND 5, when survival was 98, 66, and 3% for the 0, 25, and 50 mg/kg groups, respectively. Pup weight was reduced in treated groups with surviving litters. Gross dissection and histological examination of lungs revealed differences in maturation between control and treated animals on PND 0. We conclude that exposure to PFOS

late in gestation is sufficient to induce 100% pup mortality and that inhibition of lung maturation may be involved.

Rat and rabbit oral developmental toxicology studies with two perfluorinated compounds.
Case M. T., York R. G. and Christian M. S.
Int J Toxicol. 2001;20(2):101-9.

Developmental toxicology (teratology) studies were done on two perfluorinated compounds-perfluorooctanesulfonate (PFOS) and 2-(N-ethylperfluorooctanesulfonamido)ethyl alcohol (N-EtFOSE) in rats and rabbits. Dose selection for these oral developmental toxicity studies were based upon dose-range study results. Dose levels of 0, 1, 5, 10, and 20 mg/kg/day were used for the rat N-EtFOSE study, and dose levels of 0, 0.1, 1.0, 2.5, and 3.75 mg/kg/day were used for both the PFOS and the N-EtFOSE rabbit studies. Although no compound-related deaths occurred in the dosed pregnant females on the developmental toxicity studies, maternal toxicity (reduced body weight gain and feed consumption) was present at higher dose levels in all three studies. At high maternally toxic doses, associated effects occurred in the conceptuses--increased abortions in PFOS and N-EtFOSE rabbits, reduced fetal weights in N-EtFOSE rats and PFOS rabbits, and increased late resorptions in N-EtFOSE rabbits. Detailed external gross, soft tissue, and skeletal fetal examinations failed to reveal any compound-related malformations in either species. Similar results, that is, only effects associated with maternal toxicity, had been found in previously conducted PFOS rat developmental toxicity studies. It was concluded that these perfluorinated compounds were not selective developmental toxicants in either rats or rabbits.

B. Studies reporting no developmental or reproductive toxicity

In utero exposure to perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) did not increase body weight or intestinal tumorigenesis in multiple intestinal neoplasia (Min/+) mice.
Ngo H. T., Hetland R. B., Sabaredzovic A., Haug L. S. and Steffensen I. L.
Environ Res. 2014;132:251-63.

We examined whether perfluorooctanoate (PFOA) or perfluorooctane sulfonate (PFOS) had obesogenic effects and if they increased spontaneous intestinal tumorigenesis in the mouse model C57BL/6J-Min/+ (multiple intestinal neoplasia) after in utero exposure.

The dams were exposed to PFOA or PFOS (0.01, 0.1 or 3.0mg/kg bw/day) by po gavage on GD1-17. The Min/+ and wild-type offspring were terminated at week 11 for examination of intestinal tumorigenesis or at week 20 for obesogenic effect, respectively. Body weights of the dams and pups were recorded throughout life. Food intake was determined at week 6 and 10. Blood glucose (non-fasted) was measured at week 6 and 11. No obesogenic effect of PFOA or PFOS was observed up to 20 weeks of age. PFOA or PFOS did not increase the incidence or number of tumors in the small intestine or colon of the Min/+ mice or affect their location along the intestines. Feed intake was not affected. There were some indications of toxicity of PFOA, but not of PFOS. There was lower survival of pups after 3.0mg/kg PFOA, lower body weight in pups after 3.0 and possibly 0.1mg/kg PFOA, and increased relative liver weight after 0.01 and possibly 0.1mg/kg PFOA. Plasma glucose was lower after 0.01 and 0.1mg/kg PFOA. In conclusion, exposure to PFOA and PFOS in utero with the doses used did not have obesogenic effect on either Min/+ or wild-type mice, at least not up to 11 or 20 weeks of age, nor increased intestinal tumorigenesis in Min/+ mice.

C. Related articles

Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in aberrant changes in miRNA expression profile and levels in developing rat livers.
Wang F., Liu W., Jin Y., Wang F. and Ma J.
Environ Toxicol. 2015;30(6):712-23.

Perfluorooctane sulfonate (PFOS) is an animal carcinogen. However, the underlying mechanism in cancer initiation is still largely unknown. Recently identified microRNAs (miRNAs) may play an important role in toxicant exposure and in the process of toxicant-induced tumorigenesis. We used PFOS to investigate PFOS-induced changes in miRNA expression in developing rat liver and the potential mechanism of PFOS-induced toxic action. Dams received 3.2 mg/kg PFOS in their feed from gestational day 1 (GD1) to postnatal day 7 (PND 7). Pups then had free access to treated feed until PND 7. We isolated RNAs from liver tissues on PND 1 and 7 and analyzed the expression profiles of 387 known rat miRNAs using microarray technology. PFOS exposure induced significant changes in miRNA expression profiles. Forty-six miRNAs had significant expression alterations on PND 1, nine miRNAs on PND 7. Specifically, expression of four miRNAs was up-regulated on PND 7 but down-regulated on PND1 ($p < 0.05$). Many aberrantly expressed miRNAs were related to various cancers. We found oncogenic and tumor-suppressing miRNAs, which included miR-19b, miR-21*, miR-17-3p, miR-125a-3p, miR-16, miR-26a, miR-1, miR-200c, and miR-451. In addition, four

miRNAs were simultaneous significantly expressed on both PND 1 and 7. Functional Annotation analysis of the predicted transcript targets revealed that PFOS exposure potentially alters pathways associated with different cancers (cancer, melanoma, pancreatic cancer, colorectal cancer, and glioma), biological processes which include positive regulation of apoptosis and cell proliferation. Results showed PFOS exposure altered the expression of a suite of miRNAs. (c) 2014 Wiley Periodicals, Inc. Environ Toxicol 30: 712-723, 2015.

Possible role of serotonin and neuropeptide Y on the disruption of the reproductive axis activity by perfluorooctane sulfonate.
Lopez-Doval S., Salgado R., Fernandez-Perez B. and Lafuente A.
Toxicol Lett. 2015;233(2):138-47.

Perfluorooctane sulfonate (PFOS) is an endocrine disruptor, whose exposure can induce several alterations on the reproductive axis activity in males during adulthood. This study was undertaken to evaluate the possible role of serotonin and neuropeptide Y (NPY) on the disruption of the hypothalamic-pituitary-testicular (HPT) axis induced by PFOS in adult male rats. For that, adult male rats were orally treated with 0.5; 1.0; 3.0 and 6.0mg of PFOS/kg/day for 28 days. After PFOS exposure, serotonin concentration increased in the anterior and mediobasal hypothalamus as well as in the median eminence. The metabolism of this amine (expressed as the ratio 5-hydroxyindolacetic acid (5-HIAA)/serotonin) was diminished except in the anterior hypothalamus, with the doses of 3.0 and 6.0mg/kg/day, being this dose 0.5mg/kg/day in the median eminence. In general terms, PFOS-treated rats presented a decrease of the hypothalamic concentration of the gonadotropin releasing hormone (GnRH) and NPY. A diminution of the serum levels of the luteinizing hormone (LH), testosterone and estradiol were also shown. These results suggest that both serotonin and NPY could be involved in the inhibition induced by PFOS on the reproductive axis activity in adult male rats.

Prenatal exposure to the contaminant perfluorooctane sulfonate elevates lipid peroxidation during mouse fetal development but not in the pregnant dam.
Lee Y. Y., Wong C. K., Oger C., Durand T., Galano J. M. and Lee J. C.
Free Radic Res. 2015;49(8):1015-25.

Perfluorooctane sulfonate (PFOS), a member of the perfluorinated chemical family, has been convincingly demonstrated to affect lipid metabolism in animals and humans and readily crosses the placenta to exert its effects on the developing fetuses. While its exact mechanism is still not clear, PFOS exposure has long been suggested to exert its toxicity via oxidative stress and/or altered gene expression. Levels of PFOS and

malondialdehyde in various organs and cell cultures have been widely determined as general indicators of non-specific lipid peroxidation after PFOS exposure. In this study, the oxidation of precise polyunsaturated fatty acids and their metabolites, derived from enzymatic and non-enzymatic pathways was determined following PFOS exposure in both adult and maternal/fetal mice. CD-1 mice were exposed to 3 mg/kg body weight/day of PFOS in corn oil by oral gavage until late gestation (GD17). We demonstrated that lipid peroxidation was particularly and exclusively affected in fetuses exposed to PFOS, but this was not the case in the maternal mice, where limited effects were observed in the enzymatic oxidation pathway. In this study, we demonstrated that PFOS-induced lipid peroxidation might have a greater impact in free radical generation in fetuses than in dams and could be responsible for affecting fetal development. In addition, antioxidant enzymes, such as superoxide dismutase and catalase, appeared to maintain oxidative stress homeostasis partially in adult mice exposed to PFOS. Taken together, our results might elucidate the mechanism of how PFOS induces oxidative stress in vivo.

Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal Leydig cells.
Zhao B., Li L., Liu J., Li H., Zhang C., Han P., Zhang Y., Yuan X., Ge R. S. and Chu Y. PLoS One. 2014;9(1):e78888.

BACKGROUND: Perfluorooctane sulfonate (PFOS) is a synthetic material that has been widely used in industrial applications for decades. Exposure to PFOS has been associated with decreased adult testosterone level, and Leydig cell impairment during the time of adulthood. However, little is known about PFOS effects in utero on fetal Leydig cells (FLC). METHODS AND RESULTS: The present study investigated effects of PFOS on FLC function. Pregnant Sprague Dawley female rats received vehicle (0.05% Tween20) or PFOS (5, 20 mg/kg) by oral gavage from gestational day (GD) 11-19. At GD20, testosterone (T) production, FLC numbers and ultrastructure, testicular gene and protein expression levels were examined. The results indicate that exposures to PFOS have affected FLC function as evidenced by decreased T production, impaired FLC, reduced FLC number, and decreased steroidogenic capacity and cholesterol level in utero. CONCLUSION: The present study shows that PFOS is an endocrine disruptor of male reproductive system as it causes reduction of T production and impairment of rat fetal Leydig cells.

Chronic effects of PFOA and PFOS on sexual reproduction of freshwater rotifer Brachionus calyciflorus.
Zhang L., Niu J., Wang Y., Shi J. and Huang Q.
Chemosphere. 2014;114:114-20.

Rotifers play an important role in the dynamics of freshwater and coastal marine ecosystems, and are also important tools for assessing toxicity in aquatic environments. In this study, the effects of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) on the population growth rate and resting egg production of rotifer Brachionus calyciflorus were investigated. Reproductive bioassays indicated that PFOS increased the rotifer population growth rate at the concentration 2.0 mg L(-1), and inhibited it at higher concentrations. For PFOA, the inhibition of population growth rate was observed when the concentration was greater than 4.0 mg L(-1). Exposure to PFOS (0.25 mg L(-1)) or PFOA (2.0 mg L(-1)) increased the mictic ratios of unexposed rotifer offspring. Population variation and increased mictic ratios were likely the two major factors leading to decline of resting egg production. The resting eggs formed under exposure to PFOA/PFOS in the range of 0.125-2.0 mg L(-1) showed higher hatching percentages in the control medium than that without PFOA/PFOS exposure. When the resting eggs were formed in the control medium and incubated in media with different levels of PFOA/PFOS, higher hatching percentages were induced by PFOS but lower hatching percentages induced by PFOA. The effects on the hatching rate of resting eggs with PFOA/PFOS exposure during the hatching period were greater than those with exposure during resting egg formation period, and the effect of PFOS was greater than that of PFOA. Both PFOA and PFOS exhibited slight effect on the hatching pattern.

Perinatal exposure to perfluorooctane sulfonate affects glucose metabolism in adult offspring.
Wan H. T., Zhao Y. G., Leung P. Y. and Wong C. K.
PLoS One. 2014;9(1):e87137.

Perfluoroalkyl acids (PFAAs) are globally present in the environment and are widely distributed in human populations and wildlife. The chemicals are ubiquitous in human body fluids and have a long serum elimination half-life. The notorious member of PFAAs, perfluorooctane sulfonate (PFOS) is prioritized as a global concerning chemical at the Stockholm Convention in 2009, due to its harmful effects in mammals and aquatic organisms. PFOS is known to affect lipid metabolism in adults and was found to be able to cross human placenta. However the effects of in utero exposure to the susceptibility

of metabolic disorders in offspring have not yet been elucidated. In this study, pregnant CD-1 mice (F0) were fed with 0, 0.3 or 3 mg PFOS/kg body weight/day in corn oil by oral gavage daily throughout gestational and lactation periods. We investigated the immediate effects of perinatal exposure to PFOS on glucose metabolism in both maternal and offspring after weaning (PND 21). To determine if the perinatal exposure predisposes the risk for metabolic disorder to the offspring, weaned animals without further PFOS exposure, were fed with either standard or high-fat diet until PND 63. Fasting glucose and insulin levels were measured while HOMA-IR index and glucose AUCs were reported. Our data illustrated the first time the effects of the environmental equivalent dose of PFOS exposure on the disturbance of glucose metabolism in F1 pups and F1 adults at PND 21 and 63, respectively. Although the biological effects of PFOS on the elevated levels of fasting serum glucose and insulin levels were observed in both pups and adults of F1, the phenotypes of insulin resistance and glucose intolerance were only evident in the F1 adults. The effects were exacerbated under HFD, highlighting the synergistic action at postnatal growth on the development of metabolic disorders.

Perfluorooctanesulfonate (PFOS) perturbs male rat Sertoli cell blood-testis barrier function by affecting F-actin organization via p-FAK-Tyr(407): an in vitro study.
Wan H. T., Mruk D. D., Wong C. K. and Cheng C. Y.
Endocrinology. 2014;155(1):249-62.

Environmental toxicants such as perfluorooctanesulfonate (PFOS) have been implicated in male reproductive dysfunction, including reduced sperm count and semen quality, in humans. However, the underlying mechanism(s) remains unknown. Herein PFOS at 10-20 muM (approximately 5-10 mug/mL) was found to be more potent than bisphenol A (100 muM) in perturbing the blood-testis barrier (BTB) function by disrupting the Sertoli cell tight junction-permeability barrier without detectable cytotoxicity. We also delineated the underlying molecular mechanism by which PFOS perturbed Sertoli cell BTB function using an in vitro model that mimics the BTB in vivo. First, PFOS perturbed F-actin organization in Sertoli cells, causing truncation of actin filaments at the BTB. Thus, the actin-based cytoskeleton was no longer capable of supporting the distribution and/or localization of actin-regulatory and adhesion proteins at the cell-cell interface necessary to maintain BTB integrity. Second, PFOS was found to perturb inter-Sertoli cell gap junction (GJ) communication based on a dye-transfer assay by down-regulating the expression of connexin-43, a GJ integral membrane protein. Third, phosphorylated focal adhesion kinase (FAK)-Tyr(407) was found to protect the BTB from the destructive effects of PFOS as shown in a study via an overexpression of an FAK Y407E phosphomimetic mutant. Also, transfection of Sertoli

cells with an FAK-specific microRNA, miR-135b, to knock down the expression of phosphorylated FAK-Tyr(407) was found to worsen PFOS-mediated Sertoli cell tight junction disruption. In summary, PFOS-induced BTB disruption is mediated by down-regulating phosphorylated FAK-Tyr(407) and connexin-43, which in turn perturbed F-actin organization and GJ-based intercellular communication, leading to mislocalization of actin-regulatory and adhesion proteins at the BTB.

Evolutionary ecotoxicology of perfluoralkyl substances (PFASs) inferred from multigenerational exposure: a case study with Chironomus riparius (Diptera, Chironomidae).
Stefani F., Rusconi M., Valsecchi S. and Marziali L.
Aquat Toxicol. 2014;156:41-51.

A multigeneration toxicity test on Chironomus riparius was performed with the aim of investigating the evolutionary consequences of exposure to perfluoralkyl substances (perfluorooctane sulfonic acid, PFOS; perfluorooctanoic acid, PFOA; perfluorobutane sulfonate, PFBS). Six-hundred larvae were bred per treatment and per generation until emergence and egg deposition under a nominal concentration of 10mug/L of contaminants. Newborn larvae were used to start the next generation. Evolution of genetic variability was evaluated along a total of 10 consecutive generations based on 5 microsatellite loci. Analysis of life-history traits (survival, sex ratio and reproduction) was also carried out. Rapid genetic variability reduction was observed in all treatments, including controls, across generations due to the test conditions. Nevertheless, an increased mutation rate determined a stronger conservation of genetic variability in PFOS and, at minor extent, in PFBS exposed populations compared to controls. No significant effects were induced by exposure to PFOA. Direct mutagenicity or induced stress conditions may be at the base of increased mutation rate, indicating the potential risk of mutational load caused by exposure to PFOS and PFBS. The test provided the opportunity to evaluate the use of approximate Bayesian computation (ABC) and coalescent approaches in evolutionary ecotoxicology. A weak performance was evidenced for ABC, either in terms of bias or dispersion of effective population sizes and of estimates of mutation rate. On the contrary, coalescent simulations proved the sensitivity of traditional genetic endpoints (i.e. heterozygosity and number of alleles) to the alteration of mutation rate, but not to erosion of genetic effective size.

Chronic exposure to perfluorinated compounds: Impact on airway hyperresponsiveness and inflammation.
Ryu M. H., Jha A., Ojo O. O., Mahood T. H., Basu S., Detillieux K. A., Nikoobakht N., Wong C. S., Loewen M., Becker A. B. and Halayko A. J.
Am J Physiol Lung Cell Mol Physiol. 2014;307(10):L765-74.

Emerging epidemiological evidence reveals a link between lung disease and exposure to indoor pollutants such as perfluorinated compounds (PFCs). PFC exposure during critical developmental stages may increase asthma susceptibility. Thus, in a murine model, we tested the hypothesis that early life and continued exposure to two ubiquitous household PFCs, perfluorooctanoic acid (PFOA) and perflurooctanesulfonic acid (PFOS), can induce lung dysfunction that exacerbates allergen-induced airway hyperresponsiveness (AHR) and inflammation. Balb/c mice were exposed to PFOA or PFOS (4 mg/kg chow) from gestation day 2 to 12 wk of age by feeding pregnant and nursing dams, and weaned pups. Some pups were also sensitized and challenged with ovalbumin (OVA). We assessed lung function and inflammatory cell and cytokine expression in the lung and examined bronchial goblet cell number. PFOA, but not PFOS, without the OVA sensitization/challenge induced AHR concomitant with a 25-fold increase of lung macrophages. PFOA exposure did not affect OVA-induced lung inflammatory cell number. In contrast, PFOS exposure inhibited OVA-induced lung inflammation, decreasing total cell number in lung lavage by 68.7%. Interferon-gamma mRNA in the lung was elevated in all PFC-exposed groups. Despite these effects, neither PFOA nor PFOS affected OVA-induced AHR. Our data do not reveal PFOA or PFOS exposure as a risk factor for more severe allergic asthma-like symptoms, but PFOA alone can induce airway inflammation and alter airway function.

Chronic PFOS exposure alters the expression of neuronal development-related human homologues in Eisenia fetida.
Mayilswami S., Krishnan K., Megharaj M. and Naidu R.
Ecotoxicol Environ Saf. 2014;110:288-97.

PFOS is a toxic, persistent environmental pollutant which is widespread worldwide. PFOS contamination has entered the food chain and is interfering with normal development in man and is neurotoxic, hepatotoxic and tumorigenic. The earthworm, Eisenia fetida is one of the organisms which can help to diagnose soil health and contamination at lower levels in the food chain. Studying the chronic effects of sub-lethal PFOS exposure in such an organism is therefore appropriate. As PFOS

bioaccumulates and is not easily biodegraded, it is biomagnified up the food chain. Gene expression studies will give us information to develop biomarkers for early diagnosis of soil contamination, well before this contaminant passes up the food chain. We have carried out mRNA sequencing of control and chronically PFOS exposed E. fetida and reconstructed the transcripts in silico and identified the differentially expressed genes. Our findings suggest that PFOS up/down regulates neurodegenerative-related human homologues and can cause neuronal damage in E. fetida. This information will help to understand the links between neurodegenerative disorders and environmental pollutants such as PFOS. Furthermore, these up/down regulated genes can be used as biomarkers to detect a sub-lethal presence of PFOS in soil. Neuronal calcium sensor-2, nucleoside diphosphate kinase, polyadenylate-binding protein-1 and mitochondrial Pyruvate dehydrogenase protein-X component, could be potential biomarkers for sub lethal concentrations of PFOS.

Exposure and effects of perfluoroalkyl substances in tree swallows nesting in Minnesota and Wisconsin, USA.
Custer C. M., Custer T. W., Dummer P. M., Etterson M. A., Thogmartin W. E., Wu Q., Kannan K., Trowbridge A. and McKann P. C.
Arch Environ Contam Toxicol. 2014;66(1):120-38.

The exposure and effects of perfluoroalkyl substances (PFASs) were studied at eight locations in Minnesota and Wisconsin between 2007 and 2011 using tree swallows (Tachycineta bicolor). Concentrations of PFASs were quantified as were reproductive success end points. The sample egg method was used wherein an egg sample is collected, and the hatching success of the remaining eggs in the nest is assessed. The association between PFAS exposure and reproductive success was assessed by site comparisons, logistic regression analysis, and multistate modeling, a technique not previously used in this context. There was a negative association between concentrations of perfluorooctane sulfonate (PFOS) in eggs and hatching success. The concentration at which effects became evident (150-200 ng/g wet weight) was far lower than effect levels found in laboratory feeding trials or egg-injection studies of other avian species. This discrepancy was likely because behavioral effects and other extrinsic factors are not accounted for in these laboratory studies and the possibility that tree swallows are unusually sensitive to PFASs. The results from multistate modeling and simple logistic regression analyses were nearly identical. Multistate modeling provides a better method to examine possible effects of additional covariates and assessment of models using Akaike information criteria analyses. There was a credible association between PFOS concentrations in plasma and eggs, so extrapolation between these two commonly sampled tissues can be performed.

Effects of perfluorooctanesulfonate and perfluorobutanesulfonate on the growth and sexual development of Xenopus laevis.
Lou Q. Q., Zhang Y. F., Zhou Z., Shi Y. L., Ge Y. N., Ren D. K., Xu H. M., Zhao Y. X., Wei W. J. and Qin Z. F.
Ecotoxicology. 2013;22(7):1133-44.

Perfluorobutanesulfonate (PFBS), as a substitute for perfluorooctanesulfonate (PFOS), is widespread in the environment and biotic samples as well as PFOS. To investigate effects of PFOS and PFBS on the growth and sexual development of amphibians, we exposed Xenopus laevis tadpoles at a series of concentrations of PFOS and PFBS (0.1; 1; 100; 1,000 mug/l) as well as 17-beta-estradiol (E2, 100 ng/l) and 5 alpha-androstan-17-beta-ol-3-one (DHT, 100 ng/l) from stage 46/47 to 2 months postmetamorphosis. We found that neither PFOS nor PFBS had a significant effect on the survival and growth. However, they caused hepatohistological impairment at higher concentrations (100; 1,000 mug/l). Unlike E2, PFOS at all concentrations did not alter the sex ratio and induce intersex, but caused degeneration of spermatogonia in testes except for the lowest concentration. PFBS had no effect on the sex ratio and gonadal histology. PFOS and PFBS promoted expression of estrogen receptor (ER) and androgen receptor (AR), but not affected aromatase expression in the brain. The increase in expression of ER and AR suggests an increase in the responsiveness to the corresponding sex hormone and potential effects on sexual development. Our results show that PFBS as well as PFOS have adverse effects on hepato-histology and sexual development on X. laevis. Also, PFOS- and PFBS-induced increase in ER and AR expression highlights the need to further study effects of PFOS and PFBS on subsequently gonadal development, sexual dimorphism, and secondary sex characteristics in X. laevis. It is debatable that PFBS is widely used as a substitute of PFOS.

Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka.
Fang C., Huang Q., Ye T., Chen Y., Liu L., Kang M., Lin Y., Shen H. and Dong S.
Ecotoxicol Environ Saf. 2013;92:104-11.

Perfluorooctane sulfonate (PFOS) is a global pollutant that has been studied because of its health risks. PFOS has been shown to have immune toxicity. However, few studies have focused on the immune responses of fish larvae exposed to PFOS at early embryonic stages. In this study, the larvae of marine medaka (Oryzias melastigma) were evaluated for postnatal immune toxicity after embryonic exposure to PFOS (0, 1, 4 and 16mg/L) from 2 days post fertilization (dpf). The physiological indices, survival

rates, PFOS elimination kinetics, liver histology and gene transcription in the fish larvae were examined after depuration. The elimination rate constant (ke) of PFOS in the fish larvae ranged from 0.04+/-0.00 to 0.07+/-0.01d(-1). Embryonic exposure to PFOS severely compromised the postnatal survival of fish larvae after depuration. The survival rate and body width decreased in a concentration dependent manner. PFOS impaired the liver structure in the fish larvae by enlarging the cell nuclei and damaging the cell structure. To explore the toxic mechanisms that affect the immune responses, fish larvae at 27 days post hatch (dph) were exposed to lipopolysaccharides (LPS) to elicit an inflammatory response. The inflammatory response and immune-related genes were generally up-regulated in the fish larvae following embryonic exposure to 0mg/L PFOS. In contrast, the genes were all markedly down-regulated in the fish larvae following embryonic exposure to 1 and 4mg/L PFOS. These results suggest that early life exposure to PFOS could alter immunoregulation functions, leading to functional dysfunction or weakness of the immune system in fish larvae. The immunosuppression effects caused by PFOS could reduce the efficiency of immune defense mechanisms and increase the susceptibility to infectious agents, which may contribute to various detrimental health effects in the fish larvae.

Meta-analysis of toxicity and teratogenicity of 133 chemicals from zebrafish developmental toxicity studies.
Ducharme N. A., Peterson L. E., Benfenati E., Reif D., McCollum C. W., Gustafsson J. A. and Bondesson M.
Reprod Toxicol. 2013;41:98-108.

Zebrafish developmental toxicity testing is an emerging field, which faces considerable challenges regarding data meta-analysis and the establishment of standardized test protocols. Here, we present an initial correlation study on toxicity of 133 chemicals based on data in the literature to ascertain predictive developmental toxicity endpoints. We found that the physical properties of chemicals (BCF or logP) did not fully predict lethality or developmental outcomes. Instead, individual outcomes such as pericardial edema and yolk sac edema were more reliable indicators of developmental toxicity. In addition, we ranked the chemicals based on toxicity with the Toxicological Priority Index (ToxPi) program and via a teratogenic ratio, and found that perfluorooctane sulfonate (PFOS) had the highest ToxPi score, triphenyltin acetate had the highest average ToxPi score (corrected for missing data and having more than 4 outcomes), and N-methyl-dithiocarbamate had the highest teratogenic ratio.

Gene expression profiling in fetal rat lung during gestational perfluorooctane sulfonate exposure.
Ye L., Zhao B., Yuan K., Chu Y., Li C., Zhao C., Lian Q. Q. and Ge R. S.
Toxicol Lett. 2012;209(3):270-6.

Perfluorooctane sulfonate (PFOS) is a persistent environmental contaminant found in the tissues of humans and wildlife. It has been reported that gestational exposure to PFOS causes neonatal death of rats. However, the mechanism is still unclear. In this study, we investigated the effects of gestational PFOS exposure on the gene expression profiling of fetal rat lung at pseudoglandular stage. Adult Sprague Dawley dams were dosed orally from gestational day 12-18 with 0 (control), 5 mg/kg/day or 20 mg/kg/day PFOS. Animals were euthanized on day 18.5, fetal lung samples were collected for histochemical staining and RNA profiling analysis. PFOS did not cause apparent microscopic changes of fetal lungs. Gene expression profiling revealed that PFOS dose-dependently up-regulated the expression of 21 (5 mg/kg) and 43 (20 mg/kg) genes. These genes include five PPARalpha target genes (Acot1, Hmgcs2, Fabp4, Fabp1 and Myh7), and 4 of them are involved in lipid metabolism. The other genes were primarily included in the categories of cytoskeletal structure (Tpm1, Tnnt2, Actn3, Myoz2, Tmod1, and Mfap5), extracellular matrix (Ckm, Lum, Tnnc1, Art3, Dcn, Col17a1, Aspn, Ctsk, Itm2a, Spock2 and Orm1), transporting (Cox8h, Cox6a2 and Scnn1a) and secreted proteins (Scgb3a1, Nppb and Spp1). Our study demonstrates that in utero PFOS exposure resulted in the alteration of a set of genes which are involved in significant cytoskeletal, extracellular matrix remodeling, lipid metabolism and secreted proteins in the fetal rat lung.

Prenatal and neonatal exposure to perfluorooctane sulfonic acid results in changes in miRNA expression profiles and synapse associated proteins in developing rat brains.
Wang F., Liu W., Ma J., Yu M., Jin Y. and Dai J.
Environ Sci Technol. 2012;46(12):6822-9.

We previously identified a number of perfluorooctane sulfonic acid (PFOS)-responsive transcripts in developing rat brains using microarray analysis. However, the underlying mechanisms and functional consequences remain unclear. We hypothesized that microRNAs (miRNAs), which have emerged as powerful negative regulators of mRNA and protein levels, might be responsible for PFOS-induced mRNA changes and consequent neural dysfunctions. We used eight miRNA arrays to profile the expression

of brain miRNAs in neonatal rats on postnatal days (PND) 1 and 7 with maternal treatment of 0 (Control) and 3.2 mg/kg of PFOS feed from gestational day 1 to PND 7, and subsequently examined six potentially altered synapse-associated proteins to evaluate presumptive PFOS-responsive functions. Twenty-four brain miRNAs on PND 1 and 17 on PND 7 were significantly altered with PFOS exposure ($P < 0.05$), with miR-466b, -672, and -297, which are critical in neurodevelopment and synapse transmission, showing a more than 5-fold reduction. Levels of three synapse-involved proteins, NGFR, TrkC, and VGLUT2, were significantly decreased with no protein up-regulated on PND 1 or 7. Perfluorooctane sulfonic acid might affect calcium actions during synapse transmission in the nervous system by interfering with SYNJ1, ITPR1, and CALM1 via their targeting miRNAs. Our results indicated that miRNA had little direct regulatory effect on the expression of mRNAs and synapse-associated proteins tested in the developing rat brain exposed to PFOS, and it seems that the PFOS-induced synaptic dysfunctions and changes in transcripts resulted from a combinatory action of biological controllers and processes, rather than directed by one single factor.

Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model.
Loccisano A. E., Campbell J. L., Jr., Butenhoff J. L., Andersen M. E. and Clewell H. J., 3rd
Reprod Toxicol. 2012;33(4):452-67.

Perfluoroalkyl acid carboxylates and sulfonates (PFAAs) have many consumer and industrial applications. The persistence and widespread distribution of PFAAs have brought them under intense scrutiny. Limited PK data for PFAAs is available for humans; however, toxicological and pharmacokinetic data exist for rats, which can be useful for cross-species extrapolation. In this work, PBPK models were developed for adult male and female rats to describe the pharmacokinetics of PFOA and PFOS. The models contain a description of saturable renal resorption, free fraction of chemical in plasma, and saturable binding in liver. Both male and female rat models for each chemical were consistent with available PK data resulting from IV, oral, and dietary dosing regimens. Predicted plasma concentration curves followed trends observed in experimental data, and model predictions were within a factor of two of experimental values. PFOA and PFOS rat model output is sensitive to parameters governing renal resorption, indicating that renal resorption is responsible for the long-half life. These models, along with the PFAA gestation and lactation models published in this issue, will help address concerns about possible health effects due to PFAA exposure in the fetus and neonate and will be useful in comparing PK across life stages.

Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model.
Loccisano A. E., Campbell J. L., Jr., Butenhoff J. L., Andersen M. E. and Clewell H. J., 3rd
Reprod Toxicol. 2012;33(4):468-90.

Perfluoroalkyl carboxylates and sulfonates (PFAAs) have many consumer and industrial applications. Developmental toxicity studies in animals have raised concern about potential developmental effects of PFOA and PFOS in humans. We have developed PBPK models for PFAAs in the rat to help define a relationship between external dose, internal tissue concentrations, and observed adverse effects, and to understand how physiological changes that occur during gestation and lactation affect tissue distribution of PFAAs in the mother, fetus, and neonate. The models developed here expand upon a PBPK model for PFAAs in the adult female rat, and are consistent with available PK data. These models, along with the adult rat PFAA models, published in the companion paper, will help address concerns about possible health effects due to PFAA exposure in the fetus and neonate and will be useful in comparing PK across life stages.

Inflammation-like glial response in rat brain induced by prenatal PFOS exposure.
Zeng H. C., Zhang L., Li Y. Y., Wang Y. J., Xia W., Lin Y., Wei J. and Xu S. Q.
Neurotoxicology. 2011;32(1):130-9.

Numerous studies have indicated the neurotoxicity of perfluorooctane sulfonate (PFOS), a persistent and bioaccumulative compound, particularly during developmental stages of higher organisms. To explore the pro-inflammatory effect in the developmental neurotoxicity, effects of prenatal exposure to PFOS on glial activation in hippocampus and cortex were examined in offspring rats. Dams received 0.1, 0.6 and 2.0mg/kg bw PFOS by gavage from gestational day 2 (GD2) to GD21. Astrocyte activation markers, glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B (S-100beta) in hippocampus and cortex were both upregulated on postnatal day 0 (PND0) or PND21. In addition, the astrocyte activation was accompanied with the elevation of pro-inflammatory cytokines interleukin (IL-1beta) and tumor necrosis factor (TNF)-alpha. The mRNA levels of pro-inflammatory transcription factors, including activation protein-1 (AP-1), nuclear factor-kappaB (NF-kappaB), and cAMP response element-binding protein (CREB) were also increased, at least in the 2.0mg/kg group. In addition to the inflammatory response, two synaptic proteins, synapsin 1 (Syn1) and synaptophysin (Syp) were reduced in cortex on PND0 and PND21. In hippocampus, the Syn1 were also reduced, while the Syp were increased in cortex on either PND0 or PND21.

Obtained results indicated chronic glial activation with coexisting inflammatory and synapse injury features as a new mechanism of PFOS developmental neurotoxicity, and enhanced expression of AP-1, NF-kappaB and CREB may contributed to the adverse effect.

Interaction of PFOS and BDE-47 co-exposure on thyroid hormone levels and TH-related gene and protein expression in developing rat brains.
Wang F., Liu W., Jin Y., Dai J., Zhao H., Xie Q., Liu X., Yu W. and Ma J.
Toxicol Sci. 2011;121(2):279-91.

Perfluorooctane sulfonate (PFOS) and 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) are two persistent environmental contaminants that are toxic to developing nervous systems, particularly via their disruption of thyroid hormone (TH) function. To investigate whether an interaction existed between PFOS and BDE-47 on TH-mediated pathways, adult female Wistar rats were exposed to 3.2 and 32 mg/kg of PFOS or BDE-47 in their diet and co-exposed to a combination of each chemical (3.2 mg/kg) from gestational day 1 to postnatal day (PND) 14. Serum and brain tissues from both male and female neonates were collected on PNDs 1, 7, and 14 to examine TH-regulated gene and protein expression. The results revealed that (1) a significant accumulation difference occurred between the two chemicals; (2) On a equimolar basis, BDE-47 and PFOS affected serum total triiodothyronine and total thyroxine differently in adults and offspring; (3) there were region-specific and exposure- and time-dependent alterations in TH concentrations and tested gene and protein expression levels; and (4) interaction for the combined chemicals was only observed for brain-derived neurotrophic factor (BDNF), which exhibited a synergistic effect on PND 1 in the cortex and an antagonistic effect on PND 14 in the hippocampus. Our results suggest a complex TH-mediated gene and protein response to BDE-47 and/or PFOS exposure that seems little related to TH homeostasis and that little combined interaction of co-exposures was observed except on BDNF. The underlying mechanisms remain uncertain but seem to involve more actions than just TH-regulated pathway.

Technical-grade perfluorooctane sulfonate alters the expression of more transcripts in cultured chicken embryonic hepatocytes than linear perfluorooctane sulfonate.
O'Brien J. M., Austin A. J., Williams A., Yauk C. L., Crump D. and Kennedy S. W.
Environ Toxicol Chem. 2011;30(12):2846-59.

Recently it was discovered that the perfluorooctane sulfonate (PFOS) detected in wildlife, such as fish-eating birds, had a greater proportion of linear PFOS (L-PFOS)

than the manufactured technical product (T-PFOS), which contains linear and branched isomers. This suggests toxicological studies based on T-PFOS data may inaccurately assess exposure risk to wildlife. To determine whether PFOS effects were influenced by isomer content, we compared the transcriptional profiles of cultured chicken embryonic hepatocytes (CEH) exposed to either L-PFOS or T-PFOS using Agilent microarrays. At equal concentrations (10 microM), T-PFOS altered the expression of more transcripts (340, >1.5-fold change, p < 0.05) compared with L-PFOS (130 transcripts). Higher concentrations of L-PFOS (40 microM) were also less transcriptionally disruptive (217 transcripts) than T-PFOS at 10 microM. Functional analysis showed that L-PFOS and T-PFOS affected genes involved in lipid metabolism, hepatic system development, and cellular growth and proliferation. Pathway and interactome analysis suggested that genes may be affected through the RXR receptor, oxidative stress response, TP53 signaling, MYC signaling, Wnt/beta-catenin signaling, and PPARgamma and SREBP receptors. In all functional categories and pathways examined, the response elicited by T-PFOS was greater than that of L-PFOS. These data show that T-PFOS elicits a greater transcriptional response in CEH than L-PFOS alone and demonstrates the importance of considering the isomer-specific toxicological properties of PFOS when assessing exposure risk.

Inhibition of human and rat 3beta-hydroxysteroid dehydrogenase and 17beta-hydroxysteroid dehydrogenase 3 activities by perfluoroalkylated substances.
Zhao B., Hu G. X., Chu Y., Jin X., Gong S., Akingbemi B. T., Zhang Z., Zirkin B. R. and Ge R. S.
Chem Biol Interact. 2010;188(1):38-43.

Perfluoroalkylated substances (PFASs) including perfluorooctane acid (PFOA) and perfluorooctane sulfonate (PFOS) have been classified as persistent organic pollutants and are known to cause reduced testosterone production in human males. The objective of the present study was to compare the potencies of five different PFASs including PFOA, PFOS, potassium perfluorooctane sulfonate (PFOSK), potassium perfluorohexane sulfonate (PFHxSK) and potassium perfluorobutane sulfonate (PFBSK) in the inhibition of 3beta-hydroxysteroid dehydrogenase (3beta-HSD) and 17beta-hydroxysteroid dehydrogenase 3 (17beta-HSD3) activities in the human and rat testes. Human and rat microsomal enzymes were exposed to various PFASs. PFOS and PFOSK inhibited rat 3beta-HSD activity with IC(50) of 1.35 + or -0.05 and 1.77 + or -0.04 microM, respectively, whereas PFHxSK and PFBSK had no effect at concentrations up to 250 microM. All chemicals tested weakly inhibited human 3beta-HSD activity with IC(50)s over 250 microM. On the other hand, PFOS, PFOSK and PFOA inhibited human 17beta-HSD3 activity with IC(50)s of 6.02 + or - 1.02, 4.39 + or -

0.46 and 127.60 + or - 28.52 microM, respectively. The potencies for inhibition of 17beta-HSD3 activity were determined to be PFOSK>PFOS>PFOA>PFHxSK=PFBSK for human 17beta-HSD3 activity. There appears to be a species-dependent sensitivity to PFAS-mediated inhibition of enzyme activity because the IC(50)s of PFOS(K) for inhibition of rat 17beta-HSD3 activity was greater than 250 microM. In conclusion, the present study shows that PFOS and PFOSK are potent inhibitors of rat 3beta-HSD and human 17beta-HSD3 activity, and implies that inhibition of steroidogenic enzyme activity may be a contributing factor to the effects that PFASs exert on androgen secretion in the testis.

Transcriptional effects of prenatal and neonatal exposure to PFOS in developing rat brain.
Wang F., Liu W., Jin Y., Dai J., Yu W., Liu X. and Liu L.
Environ Sci Technol. 2010;44(5):1847-53.

Perfluorooctane sulfonate (PFOS), a persistent and bioaccumulative compound, is widely distributed in the environment. To explore the molecular mechanism of neonatal neurotoxic effects, we evaluated the transcriptional effects of prenatal and neonatal exposure to PFOS in developing rat brain by performing gene expression profiling in the cerebral cortex. Dams received 3.2 mg/kg PFOS in their feed from gestational day 1 (GD1) to weaning (PND 21). Pups then had free access to treated feed until PND 35. Six Illumina RatRef-12 Expression BeadChips were used to identify gene expression changes on postnatal days (PNDs) 1, 7, and 35. Significantly affected genes ($P < 0.05$) were involved in neuroactive ligand-receptor interaction, calcium signaling pathways, cell communication, long-term potentiation/depression, the cell cycle, and peroxisome proliferator-activated receptor (PPAR) signaling. To compare prenatal and lactational exposure contributions to transcriptional effects, a subset of altered genes obtained from the gene-profile study that represented neurobiological functions was analyzed using RT-PCR in a follow-up cross-foster study lasting from PND1 to 21. Prenatal and postnatal exposure to PFOS caused potential neurotoxicity as demonstrated by developmentally different global transcriptional changes. Prenatal exposure was more effective in altering expression of several genes. Also, transcriptional effects of PFOS exposure on neurodevelopment occurred primarily by disrupting the neuroendocrine system.

Alterations in tumor biomarker GSTP gene methylation patterns induced by prenatal exposure to PFOS.
Wan Y. J., Li Y. Y., Xia W., Chen J., Lv Z. Q., Zeng H. C., Zhang L., Yang W. J., Chen T., Lin Y., Wei J. and Xu S. Q.
Toxicology. 2010;274(1-3):57-64.

The adverse environmental exposure in early life may have adverse effects on animals through epigenetic aspects. The current study examined the possibility of early epigenetic alteration in PFOS-exposed rat liver. Pregnant Sprague-Dawley (SD) rats were exposed to perfluorooctane sulfonate (PFOS) at doses of 0.1, 0.6 and 2.0 mg/kg/d and 0.05% Tween 80 as control by gavage from gestation days 2 to 21. The dams were allowed to give birth and liver samples from weaned (postnatal day 21) offspring rats were analyzed for PFOS content, relative liver weight, global DNA methylation, methylation of LINE-1 regulatory region, tumor suppressor gene glutathione S-transferase pi (GSTP) and p16 promoter methylation level, as well as related genes expression level. In PFOS-exposed weaned rats, compared to the control, global DNA methylation and methylation of LINE-1 regulatory region decreased significantly only in the 2.0 mg/kg/d group. Up to 30% of critical CpG sites (+79, 81 and 84) in GSTP promoter region were methylated in the livers of PFOS-treated rats, while p16 promoter methylation was not affected. In addition, the up-regulated expression of GSTP was observed and this increase was associated with its main pathway of transcription regulation: Keap1-Nrf2/MafK. Thus, early-induced changes in critical cytosines within the GSTP gene promoter region may be a biomarker of hepatic PFOS burden, though their direct role in PFOS-induced hepatotoxicity, including its potential carcinogenic action, needs further research.

Effect of gestational and lactational exposure to perfluorooctanesulfonate on calcium-dependent signaling molecules gene expression in rats' hippocampus.
Liu X., Liu W., Jin Y., Yu W., Wang F. and Liu L.
Arch Toxicol. 2010;84(1):71-9.

Perfluorooctanesulfonate (PFOS) is an environmental contaminant found in human and animal tissues worldwide. The developing nervous system is thought to be particularly sensitive to PFOS by the fact that PFOS can cross blood-brain and placental barriers. Effect of gestational and lactational exposure to PFOS on central nervous system (CNS) in Wistar rats was investigated by the cross-foster model built with PFOS at 0 or 3.2 mg/kg food. Real-time reverse transcription-polymerase chain reaction was

employed to evaluate the gene expression of calcium-dependent signaling molecules in rats' hippocampus which are critical to the function of CNS. The expression of calcium-related signaling molecules, such as N-methyl-D-aspartate receptor subtype-2B (NR2B), calmodulin (CaM), Ca2+/calmodulin-dependent kinase II alpha (CaMKIIalpha) and cAMP-response element-binding (CREB) were increased in the PFOS exposure group at postnatal day 1 (PND 1). The decreased NR2B in the prenatal PFOS exposure group, the decreased CaM in the pre-/postnatal PFOS exposure group, the increased CaMKIIalpha in the whole-life PFOS exposure group and the increased CREB in the prenatal/whole-life PFOS exposure group was observed at PND 7. At PND 35, rats exhibited the decreased NR2B in the pre-/postnatal and the whole-life PFOS exposure group, and the decreased CaM in the postnatal PFOS group. The results indicate that perinatal exposure to PFOS during the critical period of development of the brain may have neurotoxic effect on CNS by mediating the molecules of calcium signaling pathway.

Influence of gestation, regular bleeding and intermittent exposure on blood perfluorooctane sulfonate levels in mice: potential factors inducing sex difference and affecting exposure evaluation.
Liu W., Li X., Xu L., Liu L., Jin Y., Sato I. and Tsuda S.
J Toxicol Sci. 2010;35(3):309-16.

Higher blood levels of perfluorooctane sulfonate (PFOS) in males than the females have been observed in many human biomonitoring studies, which is not well explained yet. The effects of gestation and regular bleeding on blood PFOS level in mice were investigated to evaluate the potential factors that could result in the sex difference. The mice were exposed to PFOS via drinking water at a concentration of 50 mug/l. After 6 weeks of pre-exposure and the gestation period, the blood PFOS concentrations in the gestagenic mice were significantly lower than the control non-gestagenic mice with a ratio of 0.45. Significant lower blood PFOS concentrations in the male mice treated by regular artificial bleeding were observed compared with those from the control male. However, such difference was not observed for the females. The sex difference in the effect of regular artificial bleeding on the blood PFOS level may be caused by the different accumulation and elimination rate in the female and male mice. In addition, the effect of intermittent exposure to PFOS on blood level was evaluated. Each single exposure caused a significant increase in blood PFOS level in both females and males, suggesting the acute exposure to PFOS occurred before the blood sampling, e.g. exposure to PFOS-contaminated foods or drinks, would affect the biomonitoring data to some extent depending on the background blood level. Thus serial blood monitoring is required to obtain accurate body burden.

Tissue distribution of (35)S-labelled perfluorooctane sulfonate (PFOS) in C57Bl/6 mice following late gestational exposure.
Borg D., Bogdanska J., Sundstrom M., Nobel S., Hakansson H., Bergman A., DePierre J. W., Halldin K. and Bergstrom U.
Reprod Toxicol. 2010;30(4):558-65.

Exposure of rodents in utero to perfluorooctane sulfonate (PFOS) impairs perinatal development and survival. Following intravenous or gavage exposure of C57Bl/6 mouse dams on gestational day (GD) 16 to (35)S-PFOS (12.5mg/kg), we determined the distribution in dams, fetuses (GD18 and GD20) and pups (postnatal day 1, PND1) employing whole-body autoradiography and liquid scintillation counting. In dams, levels were highest in liver and lungs. After placental transfer, (35)S-PFOS was present on GD18 at 2-3 times higher levels in lungs, liver and kidneys than in maternal blood. In PND1 pups, levels in lungs were significantly higher than in GD18 fetuses. A heterogeneous distribution of (35)S-PFOS was observed in brains of fetuses and pups, with levels higher than in maternal brain. This first demonstration of substantial localization of PFOS to both perinatal and adult lungs is consistent with evidence describing the lung as a target for the toxicity of PFOS at these ages.

Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid.
Rosen M. B., Schmid J. E., Das K. P., Wood C. R., Zehr R. D. and Lau C.
Reprod Toxicol. 2009;27(3-4):278-88.

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are environmental contaminants found in the tissues of humans and wildlife. They are activators of peroxisome proliferator-activated receptor-alpha (PPAR alpha) and exhibit hepatocarcinogenic potential in rats. PFOS and PFOA are also developmental toxicants in rodents and PFOS has been shown to induce pulmonary deficits in rat offspring. Pregnant CD-1 mice were dosed with 0, 5, or 10mg/kg PFOS from gestation days 1-17. Transcript profiling was conducted on the fetal liver and lung. Results were contrasted to data derived from a previous PFOA study. PFOS-dependent changes were primarily related to activation of PPAR alpha. No remarkable differences were found between PFOS and PFOA. Given that PPAR alpha signaling is required for neonatal mortality in PFOA-treated mice but not those exposed to PFOS, the neonatal mortality observed for PFOS may reflect functional deficits related to the physical properties of the chemical rather than to transcript alterations.

Cleft palate caused by perfluorooctane sulfonate is caused mainly by extrinsic factors.
Era S., Harada K. H., Toyoshima M., Inoue K., Minata M., Saito N., Takigawa T., Shiota K. and Koizumi A.
Toxicology. 2009;256(1-2):42-7.

Perfluorooctane sulfonate (PFOS) is found ubiquitously in the environment, and is known to cause developmental toxicity, including cleft plate (CP). The aim of the present study was to elucidate the mechanism of CP associated with in utero exposure to PFOS in mice. We first examined whether the concentration of PFOS in fetal serum was related to susceptibility to CP. We compared palatogenesis following the administration of various concentrations of PFOS to dams. We conducted histological examination on gestational day (GD) 15 and 18, and alizarin red/alcian blue staining of fetal heads on GD18. Finally, we cultured palatal shelves (PSs) of GD14 fetuses, which had not yet made contact with each other, for 48h, to examine whether the shelves maintained the ability to fuse. The incidence of CP increased from 7.3% with a fetal serum concentration of PFOS of 110.7+/-13.4microg/ml (13mg/kg) to 78.3% with 138.6+/-0.9microg/ml (20mg/kg). PFOS at 50mg/kg on GD11-15 caused CP at a rate of 6.1%, meanwhile PFOS at 20mg/kg on GD1-17 caused a CP rate of 89.3%. Failure of palatal shelf elevation was observed with 20mg/kg PFOS. PFOS at 20mg/kg on GD1-17 and 50mg/kg on GD11-15 inhibited mandibular growth to the same extent, even though the rate of CP was different. Explants exposed to PFOS 20mg/kg and Tween 20 showed 94% (34/36) and 100% (31/31) fusion, respectively. We demonstrated that increasing the oral dose of PFOS from 13 to 20mg/kg resulted in a significant increase in CP even though there was only a small increase in serum concentration of PFOS. PFOS prevented elevation of the PSs above the tongue because their growth/fusion potential was maintained. Mandibular hypoplasia did not seem to play a critical role in the pathogenesis of CP.

Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: toxicokinetics, thyroid hormone status, and related gene expression.
Chang S. C., Ehresman D. J., Bjork J. A., Wallace K. B., Parker G. A., Stump D. G. and Butenhoff J. L.
Reprod Toxicol. 2009;27(3-4):387-99.

Perfluorooctanesulfonate (PFOS), a persistent and accumulative compound, is widely distributed in humans and wildlife. Human exposure can occur early in development, as

evidenced by the detection of PFOS in umbilical cord blood and breast milk. As part of a developmental neurotoxicology study for which developmental endpoints, including those related to the developing nervous system, have been reported separately, groups of 25 pregnant Sprague Dawley rats were given daily oral doses of either vehicle control or potassium PFOS (K(+)PFOS) at 0.1, 0.3, and 1.0mg/kg-d from gestation day (GD) 0 (day positive for mating) through postnatal day (PND) 20. An additional 10 pregnant females per treatment group were treated through GD 19 and sacrificed on GD 20 in order to obtain maternal and fetal serum and tissue samples at the end of gestation. The present paper reports the results of samples of serum, liver, brain, and thyroid glands taken at various times to evaluate: (1) serum, liver, and brain PFOS concentrations by LC-MS/MS to establish the relationship between PFOS concentrations and study outcomes; (2) serum thyrotropin (TSH) concentrations by RIA; (3) thyroid follicular cell proliferation index by Ki-67 immunohistochemical staining; (4) thyroid follicle epithelial cell height and colloidal area by histomorphometric analysis; (5) selected liver mRNA transcripts by quantitative RT-PCR. PFOS concentrations in dam and pup serum, liver, and brain increased across treatment groups in approximate proportion to the proportional increases in maternal K(+)PFOS dose, and sex differences in PFOS concentrations were not apparent in pups on PND 21. In pups from K(+)PFOS maternal dose groups on PND 72, serum PFOS had decreased to about 3 and 11% of PND 21 concentrations in males and females, respectively, and liver PFOS had decreased to about 17% of PND 21 concentrations in both sexes. Liver PFOS concentrations were approximately 0.6-0.8 times serum PFOS in GD 20 fetuses, and increased to about 2-4 times serum concentrations on PND 4 and 21. GD 20 fetal and PND 4 pup brain PFOS concentrations were approximately 33% of the corresponding serum concentrations, dropping to approximately 10% by PND 21, in contrast to dam brain PFOS concentrations, which were approximately 4-9% of serum PFOS concentrations. Compared to controls, Cyp2b2 mRNA was increased (2.8-fold) in the 1.0mg/kg-d treatment-group dams on GD 20. In male pups on PND 21, Cyp4A1, ACoA, and Cyp2b2 were increased 2.1-, 1.5-, and 1.8-fold, respectively, and Cyp7A1 was decreased 3.5-fold. Serum TSH and thyroid follicular morphology were not altered by K(+)PFOS treatment. The mean number of proliferating thyroid follicular cells was increased 2.1-fold over control in GD 20 female fetuses from 1.0mg/kg-d-treated dams, yet the highest individual count was similar to that of controls (116 versus 113 in controls).

Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+PFOS) in rats: developmental neurotoxicity.
Butenhoff J. L., Ehresman D. J., Chang S. C., Parker G. A. and Stump D. G.
Reprod Toxicol. 2009;27(3-4):319-30.

Perfluorooctanesulfonate (PFOS), a persistent and bioaccumulative compound, is widely distributed in humans and wildlife. Exposure of the human fetus and neonate to PFOS can occur in utero and via the mother's milk, respectively. Developmental studies have been conducted with PFOS in the past, including some developmental neurotoxicity endpoints. The objective of this study was to evaluate the functional and morphological changes to the nervous system in rats having gestational and lactational exposures to PFOS per current test guidelines (EPA OPPTS 870.6300 and OECD 426). Female SD rats (25/dosage group) were given daily oral doses of either 0.0, 0.1, 0.3, or 1.0mg/kg-d potassium PFOS (K(+)PFOS) from gestation day (GD) 0 through postnatal day (PND) 20. Offspring were observed through PND 72 for growth, maturation, motor activity, learning and memory, acoustic startle reflex, various behavioral manifestations, and brain weight. Specimens were taken from dams, fetuses, and pups for serum and tissue PFOS concentration, thyroid status endpoints, and liver mRNA transcript analysis, and those results are reported in a companion article. No significant effect was noted on maternal health or reproductive outcomes from dosing of maternal rats with K(+)PFOS throughout gestation. Maternal body weights were statistically significantly lower in the 1.0mg/kg-d dosage group from PND 4 through the end of lactation. Offspring from K(+)PFOS-treated maternal groups did not differ significantly from controls with respect to birth weight, growth, age and weight at attainment of sexual maturation, learning and memory, acoustic startle, various behavioral endpoints, and brain weight. Male offspring from the 1.0mg/kg-d maternal treatment group displayed increased motor activity and reduced habituation on PND 17 but not on PND 13, 21, and 61. The maternal no-observed-adverse-effect-level (NOAEL) was 0.3mg/kg-d based on decreased body weights observed in lactation. The maternal dose associated with the NOAEL for male offspring was 0.3mg/kg-d based on increased motor activity and reduced habituation in the 1.0mg/kg-d maternal dose-group male offspring on PND 17. The maternal dose associated with the NOAEL for female offspring was >1.0mg/kg-d. Mean serum concentrations of PFOS reported in a companion article for the 0.3mg/kg-d group maternal rats are several hundred times higher than those reported for females in the United States general population.

Perfluorooctane sulfonate-induced changes in fetal rat liver gene expression.
Bjork J. A., Lau C., Chang S. C., Butenhoff J. L. and Wallace K. B.
Toxicology. 2008;251(1-3):8-20.

In utero exposure of laboratory rats to perfluorooctane sulfonate (PFOS, C(8)F(17)SO(3)(-)), a chemically stable surfactant that is widely disseminated in the environment and present in serum samples from wildlife and humans, is associated with decreased neonatal survival, and growth deficits as well as hepatomegaly. This hepatomegaly in newborn rats exposed to PFOS in utero resembles that observed in adults and is characterized by peroxisome proliferation and decreased liver triglycerides, both of which are suspected to be manifested through PPARalpha-mediated transcriptional regulation. The purpose of the present investigation was to determine whether these changes in metabolic status are a reflection of transcriptional changes in fetal rat liver using global gene expression array analyses. Gravid Sprague-Dawley rats were administered 3mg/kg PFOS by gavage daily from gestational day 2-20 and terminated on day 21. Although there was no treatment-related frank terata, there was a substantial effect of PFOS on the perinatal hepatic transcriptome-225 unique transcripts were identified as statistically increased and 220 decreased by PFOS exposure; few transcripts were changed by more than two-fold. Although the PPARalpha transcript (Ppara) itself was not affected, there was a significant increase in expression of gene transcripts associated with hepatic peroxisomal proliferation as well as those responsible for fatty acid activation, transport and oxidation pathways (both mitochondrial and peroxisomal). Additional metabolic pathways altered by in utero PFOS exposure were a stimulation of fetal hepatic fatty acid biosynthesis and a net reduction of Cyp7a1 transcript, which is required for bile acid synthesis. There were minimal effects on the expression of thyroid-related gene transcripts. In conclusion, gene expression analysis provides strong evidence indicating transcriptional control of the altered metabolic status of neonates following PFOS exposure in utero, much of which appears to be under the influence of a functional perinatal PPARalpha regulatory pathway.

APPENDIX: PENTACHLOROPHENOL

Pentachlorophenol (CAS# 87-86-5) is an organochlorine compound which is a restricted use pesticide and is primarily used industrially as a wood preservative.

This document presents a compilation of abstracts of articles on the developmental and reproductive toxicity of pentachlorophenol identified during our epidemiological screen and subsequent toxicological evaluation. OEHHA originally screened pentachlorophenol in 2007 but there was not sufficient human data available for pentachlorophenol to pass the screen at that time. We applied an epidemiologic data screen on pentachlorophenol in 2015. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. Pentachlorophenol passed the epidemiologic screen. We also conducted a preliminary toxicological evaluation searching for relevant studies, including animal studies.

OEHHA used the information in this document to select pentachlorophenol for presentation to the Developmental and Reproductive Toxicant Identification Committee as a possible candidate for Committee consideration. The abstracts compiled below are from epidemiologic and animal toxicity studies reporting on developmental and reproductive sequelae related to exposure to pentachlorophenol, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species).

Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Nine epidemiologic studies of pentachlorophenol reporting increased risk of adverse developmental or reproductive outcomes were identified, four of which were analytical studies of adequate quality. Two epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified. Seven related studies and three studies without an abstract were also identified.

In addition, the following animal toxicity studies were identified.

- Nineteen animal studies of pentachlorophenol and one meeting abstract reporting reproductive or developmental toxicity were identified, as well as three

studies reporting no reproductive or developmental toxicity. Twenty related articles and eleven studies without abstracts were identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

[*]Parental phenols exposure and spontaneous abortion in Chinese population residing in the middle and lower reaches of the Yangtze River.
Chen X., Chen M., Xu B., Tang R., Han X., Qin Y., Xu B., Hang B., Mao Z., Huo W., Xia Y., Xu Z. and Wang X.
Chemosphere. 2013;93(2):217-22.

Widespread use of phenols has led to ubiquitous exposure to phenols. In experimental animals, phenols increased resorptions, reduced live litter size and fetal body weights. However, there are limited epidemiological evidences of the relationships between exposure to phenols and pregnancy outcomes. We evaluated the associations between parental urinary levels of various phenols and spontaneous abortion in a Chinese population residing in the middle and lower reaches of the Yangtze River. A case-control study was conducted that included 70 case couples with medically unexplained spontaneous abortion and 180 control couples who did not have a history of spontaneous abortion and had at least one living child. Both parental urinary phenols were measured by ultra-high performance liquid chromatography-tandem mass spectrometry including bisphenol A (BPA), benzophenone-3 (BP-3), 2,3,4-trichlorophenol (2,3,4-TCP), pentachlorophenol (PCP), 4-n-octylphenol (4-n-OP) and 4-n-nonylphenol (4-n-NP). Compared with the low exposure group, there was an increased risk of spontaneous abortion with high paternal urinary PCP concentration [odds ratio (OR)=2.09, 95% Confidence Interval (CI), 1.05-4.14], and maternal exposure to 4-n-OP and alkylphenol(s) also significantly increased the risk of spontaneous abortion (OR=2.21, 95% CI, 1.02-4.80; OR=2.81, 95% CI, 1.39-5.65, respectively). Our study firstly provides the evidence that paternal PCP exposure, maternal 4-n-OP and alkylphenol(s) exposure are associated with spontaneous abortion in humans.

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

***Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length**.
Meijer L., Martijn A., Melessen J., Brouwer A., Weiss J., de Jong F. H. and Sauer P. J.
Hum Reprod. 2012;27(3):867-72.

BACKGROUND: Prenatal exposure to endocrine disruptors, like organohalogen compounds (OHCs), might be responsible for the increased aberrations in human male sexual development (hypospadias, cryptorchidism, testicular cancer and fall in sperm count) observed over the past decades. This development is established during fetal life, and reflected in sex hormone levels, testes volume and penile length post-partum. The present study investigates the correlation between prenatal OHC levels and male sexual development outcomes. METHODS AND RESULTS: Levels of eight neutral [2,2'-bis-(4-chlorophenyl)-1,1'-dichloroethene (4,4'-DDE), 2,2',4,4',5,5'-hexachlorobiphenyl, 2,2',4,4'-tetrabromodiphenyl ether (BDE)-47, -99, -100, -153, -154 and 1,2,5,6,9,10-hexabromocyclododecane, HBCDD] and four phenolic [(pentachlorophenol (PCP), 4OH-CB-107 (4-hydroxy-2,3,3',4',5-pentachlorobiphenyl), -146 and -187)] OHCs were determined in 55 maternal serum samples taken at 35 weeks of pregnancy. Eight sex development-related hormones [testosterone, free testosterone, sex hormone-binding globulin (SHBG); LH, FSH, estradiol (E(2)), free E(2) (FE(2)) and inhibin B (InhB)] were determined in their sons at 3 months of age, and testes volume and penile length at 3 and 18 months of age. The following prenatal OHC levels correlated significantly with sex hormone levels: PCP with SHBG and InhB (rho = 0.30 and -0.43, respectively), 4OH-CB-107 with testosterone (rho = 0.31) and BDE-154 with FE(2), E(2) and InhB (rho = 0.49, 0.54 and 0.34, respectively). BDE-154 levels correlated positively with testes volume at 18 months of age (rho = 0.34). CONCLUSIONS: Prenatal OHC exposure is correlated with aspects of sexual development outcome in boys up to 18 months of age.

***Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age**.
Roze E., Meijer L., Bakker A., Van Braeckel K. N., Sauer P. J. and Bos A. F.
Environ Health Perspect. 2009;117(12):1953-8.

BACKGROUND: Organohalogen compounds (OHCs) are known to have neurotoxic effects on the developing brain. OBJECTIVE: We investigated the influence of prenatal exposure to OHCs, including brominated flame retardants, on motor, cognitive, and

*denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

behavioral outcome in healthy children of school age. METHODS: This study was part of the prospective Groningen infant COMPARE (Comparison of Exposure-Effect Pathways to Improve the Assessment of Human Health Risks of Complex Environmental Mixtures of Organohalogens) study. It included 62 children in whose mothers the following compounds had been determined in the 35th week of pregnancy: 2,2'-bis-(4 chlorophenyl)-1,1'-dichloroethene, pentachlorophenol (PCP), polychlorinated biphenyl congener 153 (PCB-153), 4-hydroxy-2,3,3',4',5-pentachlorobiphenyl (4OH-CB-107), 4OH-CB-146, 4OH-CB-187, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), BDE-99, BDE-100, BDE-153, BDE-154, and hexabromocyclododecane. Thyroid hormones were determined in umbilical cord blood. When the children were 5-6 years of age, we assessed their neuropsychological functioning: motor performance (coordination, fine motor skills), cognition (intelligence, visual perception, visuomotor integration, inhibitory control, verbal memory, and attention), and behavior. RESULTS: Brominated flame retardants correlated with worse fine manipulative abilities, worse attention, better coordination, better visual perception, and better behavior. Chlorinated OHCs correlated with less choreiform dyskinesia. Hydroxylated polychlorinated biphenyls correlated with worse fine manipulative abilities, better attention, and better visual perception. The wood protective agent (PCP) correlated with worse coordination, less sensory integrity, worse attention, and worse visuomotor integration. CONCLUSIONS: Our results demonstrate for the first time that transplacental transfer of polybrominated flame retardants is associated with the development of children at school age. Because of the widespread use of these compounds, especially in the United States, where concentrations in the environment are four times higher than in Europe, these results cause serious concern.

Thyroid hormone levels of pregnant Inuit women and their infants exposed to environmental contaminants.
Dallaire R., Muckle G., Dewailly E., Jacobson S. W., Jacobson J. L., Sandanger T. M., Sandau C. D. and Ayotte P.
Environ Health Perspect. 2009;117(6):1014-20.

BACKGROUND: An increasing number of studies have shown that several ubiquitous environmental contaminants possess thyroid hormone-disrupting capacities. Prenatal exposure to some of them, such as polychlorinated biphenyls (PCBs), has also been associated with adverse neurodevelopmental effects in infants. OBJECTIVES: In this study we examined the relationship between exposure to potential thyroid hormone-disrupting toxicants and thyroid hormone status in pregnant Inuit women from Nunavik and their infants within the first year of life. METHODS: We measured thyroid hormone parameters [thyroid stimulating hormone (TSH), free thyroxine (fT(4)), total

triiodothyronine (T(3)), thyroxine-binding globulin (TBG)] and concentrations of several contaminants [PCB-153, hydroxylated metabolites of PCBs (HO-PCBs), pentachlorophenol (PCP) and hexachlorobenzene (HCB)] in maternal plasma at delivery (n = 120), in umbilical cord plasma (n = 95), and in infant plasma at 7 months postpartum (n = 130). RESULTS: In pregnant women, we found a positive association between HO-PCBs and T(3) concentrations (beta = 0.57, p = 0.02). In umbilical cord blood, PCB-153 concentrations were negatively associated with TBG levels (beta = -0.26, p = 0.01). In a subsample analysis, a negative relationship was also found between maternal PCP levels and cord fT(4) concentrations in neonates (beta = -0.59, p = 0.02). No association was observed between contaminants and thyroid hormones at 7 months of age. CONCLUSION: Overall, there is little evidence that the environmental contaminants analyzed in this study affect thyroid hormone status in Inuit mothers and their infants. The possibility that PCP may decrease thyroxine levels in neonates requires further investigation.

***Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols, and pentachlorophenol**. Guvenius D. M., Aronsson A., Ekman-Ordeberg G., Bergman A. and Noren K. Environ Health Perspect. 2003;111(9):1235-41.

The aim of this study was to determine human prenatal and postnatal exposures to polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), hydroxylated metabolites of PCBs (polychlorobiphenylols; OH-PCBs), and pentachlorophenol (PCP). The median PBDE fresh-weight concentrations in maternal and cord blood plasma and in breast milk were 24, 4.3, and 75 pg/g, respectively. The PCB concentrations were approximately 60 times higher in each compartment (1,560, 277, and 4,310 pg/g, respectively). Calculated on a lipid weight basis, the levels were comparable in maternal blood plasma and breast milk. In contrast to PCBs, differences were found between PBDE congener distribution in maternal and cord blood plasma. The OH-PCBs constituted up to 26% of the PCB levels in maternal blood plasma and 53% in cord blood plasma, with levels of 120 and 88 pg/g fresh weight, respectively, and in breast milk 3 pg/g. The corresponding concentrations for PCP were 2,830, 1,960, and 20 pg/g. The ratios of PCB to OH-PCB were 13, 3, and 1,400 in maternal, cord plasma, and breast milk, respectively. It is evident that prenatal exposures occur for all the analytes. Moreover, the exposure continues after birth via breast milk. However, levels of OH-PCBs and PCP in breast milk are low compared with levels in blood

*denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

plasma. Exposures to both PCBs and PBDEs, and in particular to the endocrine-active halogenated phenolic compounds, are of concern and implicate a potential risk for developmental disturbances.

Pentachlorophenol and hydroxylated polychlorinated biphenyl metabolites in umbilical cord plasma of neonates from coastal populations in Quebec.
Sandau C. D., Ayotte P., Dewailly E., Duffe J. and Norstrom R. J.
Environ Health Perspect. 2002;110(4):411-7.

Concentrations of polychlorinated biphenyls (PCBs), hydroxylated metabolites of PCBs (HO-PCBs) and octachlorostyrene (4-HO-HpCS), and pentachlorophenol (PCP) were determined in umbilical cord plasma samples from three different regions of Quebec. The regions studied included two coastal areas where exposure to PCBs is high because of marine-food-based diets--Nunavik (Inuit people) and the Lower North Shore of the Gulf of St. Lawrence (subsistence fishermen)--and a southern Quebec urban center where PCB exposure is at background levels (Quebec City). The main chlorinated phenolic compound in all regions was PCP. Concentrations of PCP were not significantly different among regions (geometric mean concentration 1,670 pg/g, range 628-7,680 pg/g wet weight in plasma). The ratio of PCP to polychlorinated biphenyl congener number 153 (CB153) concentration ranged from 0.72 to 42.3. Sum HO-PCB (sigma HO-PCBs) concentrations were different among regions, with geometric mean concentrations of 553 (range 238-1,750), 286 (103-788), and 234 (147-464) pg/g wet weight plasma for the Lower North Shore, Nunavik, and the southern Quebec groups, respectively. Lower North Shore samples also had the highest geometric mean concentration of sum PCBs (sum of 49 congeners; sigma PCBs), 2,710 (525-7,720) pg/g wet weight plasma. sigma PCB concentrations for Nunavik samples and southern samples were 1,510 (309-6,230) and 843 (290-1,650) pg/g wet weight plasma. Concentrations (log transformed) of sigma HO-PCBs and sigma PCBs were significantly correlated ($r = 0.62$, $p < 0.001$), as were concentrations of all major individual HO-PCB congeners and individual PCB congeners. In Nunavik and Lower North Shore samples, free thyroxine (T4) concentrations (log transformed) were negatively correlated with the sum of quantitated chlorinated phenolic compounds (sum PCP and sigma HO-PCBs; $r = -0.47$, $p = 0.01$, $n = 20$) and were not correlated with any PCB congeners or sigma PCBs. This suggests that PCP and HO-PCBs are possibly altering thyroid hormone status in newborns, which could lead to neurodevelopmental effects in infants. Further studies are needed to examine the effects of chlorinated phenolic compounds on thyroid hormone status in newborns.

Pentachlorophenol exposure in women with gynecological and endocrine dysfunction.
Gerhard I., Frick A., Monga B. and Runnebaum B.
Environ Res. 1999;80(4):383-8.

Exposure to wood preservatives containing pentachlorophenol (PCP) was detected in 65 women who consulted the Endocrinological Department of the University Hospital of Obstetrics and Gynecology, Heidelberg, Germany, because of gynecological problems. Blood PCP levels ranged from 20.7 to 133 microg per liter of serum. One hundred and six women with similar clinical conditions, corresponding age and body weight, no PCP exposure in history, and PCP levels below 20 microg per liter of serum served as control group. Significant associations were found between serum PCP concentrations, age, and different parameters of the endocrine system. PCP may act centrally on a hypothalamic or suprahypothalamic level which may result in mild ovarian and adrenal insufficiency. PCP may, therefore, play a role in the increasing infertility problem.

Chlorinated hydrocarbons in women with repeated miscarriages.
Gerhard I., Daniel V., Link S., Monga B. and Runnebaum B.
Environ Health Perspect. 1998;106(10):675-81.

This study was conducted to investigate a possible etiological role of chlorinated hydrocarbons in the pathogenesis of repeated miscarriages. The blood levels of chlorinated hydrocarbons [CHCs: pentachlorophenol, hexachlorocyclohexane, hexachlorobenzene, the dichlorodiphenyltrichloroethane (DDT) group, polychlorinated biphenyls] were determined in 89 women with repeated miscarriages, who were referred to the University Hospital of Obstetrics and Gynecology of Heidelberg for investigations between 1989 and 1993, and compared to a previously investigated reference population. In more than 20% of the women, at least one of the CHC levels exceeded the reference range. CHC levels did not differ significantly between women with primary or secondary and early or late miscarriages; neither did they differ between women with hormonal or immunological disorders as causes of repeated miscarriages or women with idiopathic repeated miscarriages. No significant associations were detected between CHC levels and further conceptions or the outcome of further pregnancies. As significant associations were found between increasing CHC blood concentrations and immunological and hormonal changes, CHCs may have an impact on the pregnancy course in certain cases.

Reduced birthweight and length in the offspring of females exposed to PCDFs, PCP, and lindane.
Karmaus W. and Wolf N.
Environ Health Perspect. 1995;103(12):1120-5.

The objective of this study was to investigate a broad range of adverse health outcomes and their potential association to wood preservative used in daycare centers. This article focuses on reproductive effects. A sample of 221 exposed teachers was provided by the employer's liability insurers. A comparison group (n = 189) insured by the same two organizations was recruited from nonexposed daycare centers. In a face-to-face interview, job history and reproductive history of 398 female teachers were ascertained. Data on exposure were provided, including measurements on concentration of pentachlorophenol (PCP) and lindane in wood panels, and of PCP, lindane, polychlorinated dibenzo-p-dioxins and dibenzofurans in indoor air. An exposure matrix based on individual job history, independent exposure information from each center, and reproductive history was set up with regard to the vulnerable time windows for each pregnancy. Using this approach, 49 exposed and 507 nonexposed pregnancies were identified, including 32 exposed and 386 nonexposed live births. For subgroup analyses the observations were restricted to independent pregnancies, excluding multiple and consecutive births. The data were analyzed with linear regression techniques, taking confounders into account. The crude median difference between exposed and nonexposed was 175 g in birthweight and 2 cm in length. Controlling for confounders, the results show a significantly reduced birth weight ($p = 0.04$) and length ($p = 0.02$) in exposed pregnancies, even after restricting the data to independent pregnancies and pregnancies for which data could be validated from the mother's health cards. These differences were not explained by differences in gestational age indicating that a toxic effect, which could cause small-for date newborns, might have affected the fetus.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Association of exposure to phenols and idiopathic male infertility.
Chen M., Tang R., Fu G., Xu B., Zhu P., Qiao S., Chen X., Xu B., Qin Y., Lu C., Hang B., Xia Y. and Wang X.
J Hazard Mater. 2013;250-251:115-21.

Widespread human exposure to phenols has been documented recently, and some phenols which are potential endocrine disruptors have demonstrated adverse effects on

male reproduction in animal and in vitro studies. However, implications about exposure to phenols and male infertility are scarce in humans. Case-control study of 877 idiopathic infertile men and 713 fertile controls was conducted. Urinary levels of bisphenol A, benzophenone-3, pentachlorophenol, triclosan, 4-tert-octylphenol (4-t-OP), 4-n-octylphenol (4-n-OP) and 4-n-nonylphenol (4-n-NP) and semen parameters were measured. After multivariate adjustment, we found 4-t-OP, 4-n-OP and 4-n-NP exposure was associated with idiopathic male infertility (p-value for trend: <0.0001, 0.014 and 0.001, respectively). Aside from these associations, 4-t-OP and 4-n-NP exposure was also associated with idiopathic male infertility with abnormal semen parameters. Moreover, we observed significant associations between sum alkylphenols (APs) exposure and idiopathic male infertility. There were no relationships between exposure to other phenols and idiopathic male infertility in the present study. Our study provides the first evidence that exposure to APs (4-t-OP, 4-n-OP and 4-n-NP) is associated with idiopathic male infertility.

In utero pesticide exposure, maternal paraoxonase activity, and head circumference.
Berkowitz G. S., Wetmur J. G., Birman-Deych E., Obel J., Lapinski R. H., Godbold J. H., Holzman I. R. and Wolff M. S.
Environ Health Perspect. 2004;112(3):388-91.

Although the use of pesticides in inner-city homes of the United States is of considerable magnitude, little is known about the potentially adverse health effects of such exposure. Recent animal data suggest that exposure to pesticides during pregnancy and early life may impair growth and neurodevelopment in the offspring. To investigate the relationship among prenatal pesticide exposure, paraoxonase (PON1) polymorphisms and enzyme activity, and infant growth and neurodevelopment, we are conducting a prospective, multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City. In this report we evaluate the effects of pesticide exposure on birth weight, length, head circumference, and gestational age among 404 births between May 1998 and May 2002. Pesticide exposure was assessed by a prenatal questionnaire administered to the mothers during the early third trimester as well as by analysis of maternal urinary pentachlorophenol levels and maternal metabolites of chlorpyrifos and pyrethroids. Neither the questionnaire data nor the pesticide metabolite levels were associated with any of the fetal growth indices or gestational age. However, when the level of maternal PON1 activity was taken into account, maternal levels of chlorpyrifos above the limit of detection coupled with low maternal PON1 activity were associated with a significant but small reduction in head circumference. In addition, maternal PON1 levels alone, but not PON1 genetic

polymorphisms, were associated with reduced head size. Because small head size has been found to be predictive of subsequent cognitive ability, these data suggest that chlorpyrifos may have a detrimental effect on fetal neurodevelopment among mothers who exhibit low PON1 activity.

C. Related articles

Serum concentrations of neutral and phenolic organohalogens in pregnant women and some of their infants in The Netherlands.
Meijer L., Weiss J., Van Velzen M., Brouwer A., Bergman A. and Sauer P. J.
Environ Sci Technol. 2008;42(9):3428-33.

As part of a large European Union (EU)-funded comparative toxicology and human epidemiology study, EU-Compare, a selection of organohalogen compounds (OHCs) was analyzed in maternal serum, collected at the 35th week of pregnancy, and in cord serum of a number of their infants to determine maternal concentrations and to investigate the extent of transplacental transfer of these compounds. Eight neutral OHCs were analyzed: one polychlorinated biphenyl (PCB: CB-153),4,4'-DDE, five polybrominated diphenyl ethers (PBDEs: BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154), and hexabromocyclododecane (HBCDD). Five phenolic OHCs were analyzed: three hydroxylated PCBs (40H-CB-107, 40H-CB-146, and 40H-CB-187), one hydroxylated PBDE (60H-BDE-47), and pentachlorophenol (PCP). All OHCs, except 60H-BDE-47, were present in maternal and cord serum. The historically identified OHCs showed the highest concentration: 4,4'-DDE (median value 89 ng/g lipid in maternal serum and 68 ng/g lipid in cord serum) and PCP (median value 970 pg/g serum in maternal serum and 1500 pg/g serum in cord serum). HBCDD and the PBDEs were present at much lower concentrations. We conclude that OHCs are present in the serum of pregnant women, and all compounds tested are transferred over the placenta. Because transfer is occurring at a critical stage of infant development, investigation of the health impact is urgent.

Measurement of pesticides and other toxicants in amniotic fluid as a potential biomarker of prenatal exposure: a validation study.
Bradman A., Barr D. B., Claus Henn B. G., Drumheller T., Curry C. and Eskenazi B.
Environ Health Perspect. 2003;111(14):1779-82.

Prenatal pesticide exposures may adversely affect children's health. However, exposure and health research is hampered by the lack of reliable fetal exposure data. No studies have been published that report measurements of commonly used nonpersistent pesticides in human amniotic fluid, although recent studies of pesticides in urine from pregnant women and in meconium indicate that fetuses are exposed to these chemicals. Amniotic fluid collected during amniocentesis is the only medium available to characterize direct fetal exposures early in pregnancy (approximately 18 weeks of gestation). As a first step in validating this exposure biomarker, we collected 100 amniotic fluid samples slated for disposal and evaluated analytical methods to measure organophosphate and carbamate pesticides and metabolites, synthetic pyrethroid metabolites, herbicides, and chlorinated phenolic compounds. The following six phenols were detected (detection frequency): 1- and 2-naphthol (70%), 2,5-dichlorophenol (55%), carbofuranphenol (5%), ortho-phenylphenol (30%), and pentachlorophenol (15%), with geometric mean concentrations of 0.72, 0.39, 0.12, 0.13, and 0.23 microg/L, respectively, for positive values. The organophosphate metabolites diethylphosphate and dimethylphosphate were detected in two (10%) samples, and dimethylthiophosphate was detected in one (5%) sample, with geometric mean concentrations of 0.31, 0.32, and 0.43 microg/L, respectively, for positive values. These levels are low compared with levels reported in urine, blood, and meconium in other studies, but indicate direct exposures to the young fetus, possibly during critical periods of development. Results of this pilot study suggest that amniotic fluid offers a unique opportunity to investigate fetal exposures and health risks.

Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort.
Berkowitz G. S., Obel J., Deych E., Lapinski R., Godbold J., Liu Z., Landrigan P. J. and Wolff M. S.
Environ Health Perspect. 2003;111(1):79-84.

Evidence is growing that indoor pesticide exposure is of considerable magnitude in the United States and that pesticide concentrations may be especially high in urban areas. Of particular concern is exposure of pregnant women because animal data suggest that exposure to pesticides during pregnancy and early life may impair neurodevelopment in the offspring. To investigate the relationship between prenatal exposure to indoor pesticides and infant growth and development, we are conducting a prospective,

multiethnic cohort study of mothers and infants delivered at Mount Sinai Hospital in New York City. This article provides data on pesticide exposure based on questionnaire items and analysis of maternal urinary metabolite levels among 386 women. Both the questionnaire and laboratory data revealed that exposure to indoor pesticides was considerable. The proportion of women estimated from questionnaire data as having been exposed during pregnancy to indoor pesticides (approximately 70%) was somewhat lower than the 80-90% of American households who reportedly used pesticides in previous surveys, but some of the latter surveys included both indoor and outdoor pesticide use. Urinary metabolite levels of 3,5,6-trichloro-2-pyridinol (TCPy; median = 11.3 micro g/g creatinine), phenoxybenzoic acid (PBA; median =19.3 micro g/g creatinine), and pentachlorophenol (PCP; median =7.3 micro g/g creatinine) were higher than those reported in other studies of adults in the United States. Furthermore, no associations were evident between the pesticide questionnaire data and the urinary metabolites. Assessments of sociodemographic and building characteristics with questionnaire data and the metabolite levels revealed no consistent trends. Significant temporal variations were observed for urinary PBA but not TCPy or PCP. The temporal variations for PBA were consistent with seasonal spraying of pyrethroid pesticides. These data underscore the need to assess the potentially adverse effects of pesticide exposure on fetuses and infants and the importance of finding alternative methods for pest management to reduce pesticide exposures.

Fetal exposure to pollutants in Townsville, Australia, detected in meconium.
Whitehall J. S., Ostrea E. M., Bolisetty S., Whitehall J. F. and Patole S. K.
Pediatr Res. 2000;47(4 pt 2).

OBJECTIVE: To identify fetal pollutants in meconium and correlate their presence with demographic data. BACKGROUND: 10% of tropical Townsville is ethnically Aboriginal and Islander (AI), prone to low birth weight (LBW) and Sudden Infant Death Syndrome (SIDS). Pesticides and smoking are common; illicit drugs not uncommon. These may harm the foetus and be detected in meconium. DESIGN/METHODS: Meconium samples from 45 infants from Townsville were analysed in Detroit by radioimmunoassay for drugs and gas chromatography/mass spectroscopy for pesticides and pollutants. Results were linked to data of race, birthweight, gestational age, miscarriages, postcode & thyroid status. RESULTS: 44 samples were screened for pesticides (mcg/mL). 35 (78%) revealed lindane. All 23 babies less than 2500 gm were positive for lindane (p0.001) & their mean levels were greater (0.2 vs 0.06) (p0.012). Pentachlorphenol was found in 19 (43%): mean 8.9 (0.61-31.86); chlorpyrifos, 26 (59%): mean 0.52 (0.01-0.46); malathion, 15 (34%): mean 0.052 (0.01-0.33); chlordane, 7 (16%): mean 0.23 (0.02-0.49); DDT, 23 (52%) mean 0.04 (0.01-0.12); polychlorinated biphenyls, 12 (27%),

mean 0.51 (0.04-2.63). No heavy metals were found. More AI were exposed to chlorpyrifos (p0.007). No other demographic correlations were found. None were hypothyroid. 45 samples were assayed for drugs: all contained cotinine. 8 babies had less than 15; 28, 15-35; 4, 35-50 & 5 greater than 50 ng/mL. AIs had more: mean 40.1 (18.7-119.3) vs 21.8 (7.1-68.39) ng/mL (p0.013). 9 (20%) samples had cannabinoids, mean: 76 (2.34-171.55) ng/mL. 18 had opiates, mean: 1258 (133.1-2794) ng/mL, all received opiates in labour. No cocaine or amphetamines were found. The median (range) number of pollutants per baby was 4 (1-8). The meconium of a 25 weeker had 7 pollutants. CONCLUSIONS: Many pollutants have been unavailable for years, but still exist in the food chain & cross the placenta. Pyrifos & malathion are still used. Though individual concentrations are low, many babies had several pollutants whose effects may be additive. Preterm exposure emphasises concerns for the growing brain. Lindane's association with low birth weight has been reported. 82% of babies had cotinine levels greater than 15 ng/mL, at least consistent with passive maternal smoking. No AI had less, and 37% had levels suggesting greater than one pack/day. Their greater rate of LBW & SIDS may reflect greater consumption of nicotine, compounded by pollutants. Cannabinoids are an added problem, with prevalence greater than in Detroit.

Environmental factors in infertility.
Hruska K. S., Furth P. A., Seifer D. B., Sharara F. I. and Flaws J. A.
Clin Obstet Gynecol. 2000;43(4):821-9.

In conclusion, several studies indicate that there is an association between cigarette smoking and adverse reproductive outcomes in women as well as men. Some studies indicate that alcohol consumption impairs the reproductive capacity of women. Exposures to PCE in the dry cleaning industry, toluene in the printing business, ethylene oxide and mixed solvents have been associated with decreased fecundity. Abnormalities in sperm production have been found in men exposed to radiant heat or heavy metals. Environmental exposure to chlorinated hydrocarbons (e.g., DDT, PCB, pentachlorophenol, hexachlorocyclohexane) has been associated with an increase in rates of miscarriage and endometriosis. Clinicians should counsel patients who are trying to achieve a successful pregnancy to stop smoking and limit alcohol intake. Clinicians can additionally counsel patients who are in contact with potentially harmful occupational and environmental toxicants to limit their exposure. It is important to recognize, however, that many of the studies to date are limited by small sample size, poor exposure assessment, poor outcome measurements, recruitment bias, or recall bias. Additional studies will be necessary to clarify the magnitude of risk associated with these factors.

Reproductive effects of paternal exposure to chlorophenate wood preservatives in the sawmill industry.
Dimich-Ward H., Hertzman C., Teschke K., Hershler R., Marion S. A., Ostry A. and Kelly S.
Scand J Work Environ Health. 1996;22(4):267-73.

OBJECTIVES: The purpose of the study was to determine whether paternal occupational exposure to dioxin-contaminated chlorophenols is associated with an increased risk of congenital anomalies or other adverse reproductive outcomes in offspring. METHODS: As a result of a multistep linkage, 19675 births between 1952 and 1988 were identified as children of a cohort of 9512 fathers who had worked at least one year in British Columbia sawmills where chlorophenate wood preservatives had been used. A nested case-referent analysis was applied, using conditional logistic regression, with five referents matched per case according to year of birth and gender. Chlorophenate exposure was based on expert raters' estimations of hours of exposure applied to specific time windows prior to birth. RESULTS: The offspring of male sawmill workers were at increased risk for developing congenital anomalies of the eye, particularly congenital cataracts; elevated risks for developing anencephaly or spina bifida and congenital anomalies of genital organs were shown according to specific windows of exposure. No associations were found for low birthweight, prematurity, stillbirths, or neonatal deaths. CONCLUSIONS: The study adds further support to the hypothesis of male-mediated developmental toxicity. Paternal exposure to chlorophenates was associated with the development of certain congenital anomalies in offspring.

Developmental toxicity and structure-activity relationships of chlorophenols using human embryonic palatal mesenchymal cells.
Zhao F., Mayura K., Hutchinson R. W., Lewis R. P., Burghardt R. C. and Phillips T. D.
Toxicol Lett. 1995;78(1):35-42.

The chlorophenols (CPs) comprise a major class of widely distributed and frequently occurring environmental contaminants. Previous studies have demonstrated the adverse effects of CPs on embryonic and fetal development. HEPM (human embryonic palatal mesenchymal) and MOT (mouse ovarian tumor) cell lines have been utilized in complementary bioassays for the detection of teratogens, but not the CPs. In this study, our objectives were 2-fold: (1) to determine if the HEPM assay could be used to complement other bioassay systems of nonhuman origin, i.e., Hydra attenuata (HA) and rat whole embryo culture (WEC), in the evaluation of the developmental toxicity of CPs, and (2) to delineate the ability of the HEPM assay to evaluate structure-activity

relationships of pentachlorophenol (C5P), 2,3,4,5-tetrachlorophenol (C4P), 2,3,5-trichlorophenol (C3P), 3,5-dichlorophenol (C2P), 4-monochlorophenol (CP), phenol, and CP derivatives (i.e., acetates, sodium phenates and anisoles). HEPM cells were seeded into each well of a 24-well plate and cultivated for 24 h. The medium was replaced with fresh medium containing various concentrations of test chemicals dissolved in dimethyl sulfoxide (DMSO, 0.1%). After culturing for 72 h, the medium was removed, cells were trypsinized, and cell number determined. The HEPM cell growth inhibition assay demonstrated a linear relationship between the IC50 values of the CPs and degree of chlorine substitution. The IC50 values of C5P, C4P, C3P, C2P, CP, and phenol were 18.8, 21.5, 27.5, 63.0, 150.0 and 470.0 microM, respectively. A clear structure-activity relationship was observed between toxicity of CPs and the degree of chlorine substitution. The rank order of CP toxicity from the HEPM assay (i.e., C5P > C4P > C3P > C2P > CP > phenol) is in excellent agreement with previous in vitro and in vivo studies. However, contrary to published reports, the HEPM assay predicted that all CPs were teratogenic (false positives). These findings suggest that the HEPM cell growth inhibition bioassay may be useful to discriminate between subtle differences in structure-activity and, in combination with other bioassays, might facilitate the rapid detection and prioritization of diverse cytotoxins, including various developmental toxicants. Importantly, conclusions about the teratogenicity of a test chemical (via HEPM testing) should be approached with caution and confirmed with other teratogen-sensitive systems.

D. Titles only (abstracts not available)

Exposure to pentachlorophenol as a possible cause of miscarriages.
de Maeyer J., Schepens P. J., Jorens P. G. and Verstraete R.
BJOG. 1995;102(12):1010-1.

Occupational exposures associated with male reproductive dysfunction.
Schrag S. D. and Dixon R. L.
Annu Rev Pharmacol Toxicol. 1985;25:567-92.

EFFECTS OF PENTACHLORONITROBENZENE,HEXACHLOROBENZENE AND RELATED COMPOUNDS ON FETAL DEVELOPMENT.
Courtney K. D., Copeland M. F. and Robbins A.
Toxicol Appl Pharmacol. 1976;35:239-56.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Sperm mitochondrial integrity is not required for hyperactivated motility, zona binding, or acrosome reaction in the rhesus macaque.
Hung P. H., Miller M. G., Meyers S. A. and VandeVoort C. A.
Biol Reprod. 2008;79(2):367-75.

Whether the main energy source for sperm motility is from oxidative phosphorylation or glycolysis has been long-debated in the field of reproductive biology. Using the rhesus monkey as a model, we examined the role of glycolysis and oxidative phosphorylation in sperm function by using alpha-chlorohydrin (ACH), a glycolysis inhibitor, and pentachlorophenol (PCP), an oxidative phosphorylation uncoupler. Sperm treated with ACH showed no change in percentage of motile sperm, although sperm motion was impaired. The ACH-treated sperm did not display either hyperactivity- or hyperactivation-associated changes in protein tyrosine phosphorylation. When treated with PCP, sperm motion parameters were affected by the highest level of PCP (200 microM); however, PCP did not cause motility impairments even after chemical activation. Sperm treated with PCP were able to display hyperactivity and tyrosine phosphorylation after chemical activation. In contrast with motility measurements, treatment with either the glycolytic inhibitor or the oxidative phosphorylation inhibitor did not affect sperm-zona binding and zona-induced acrosome reaction. The results suggest glycolysis is essential to support sperm motility, hyperactivity, and protein tyrosine phosphorylation, while energy from oxidative phosphorylation is not necessary for hyperactivated sperm motility, tyrosine phosphorylation, sperm-zona binding, and acrosome reaction in the rhesus macaque.

Developmental exposure to pentachlorophenol affects the expression of thyroid hormone receptor beta1 and synapsin I in brain, resulting in thyroid function vulnerability in rats.
Kawaguchi M., Morohoshi K., Saita E., Yanagisawa R., Watanabe G., Takano H., Morita M., Imai H., Taya K. and Himi T.
Endocrine. 2008;33(3):277-84.

Pentachlorophenol (PCP), a component of biocides and a contaminant in diverse tissue samples from humans from various geographic areas, disrupts regulatory effects of thyroid hormones. Here we examined the effects of developmental exposure of rats to

PCP on various aspects of brain development, male reproductive function, and adrenal function, all of which are under thyroid hormones regulation. PCP was administered to dams and their offspring via drinking water (6.6 mg l(-1)) during gestation and lactation. Tissue samples were obtained from dams, 3-week-old weanling pups, and 12-week-old pups. Gene expressions of thyroid hormone receptor beta1 and synapsin I, factors that promote brain growth, was increased in the cerebral cortex of PCP-treated weanling females, whereas plasma concentrations of total thyroxine were decreased in dams and weanling pups, and plasma thyroid-stimulating hormone concentrations were higher in PCP-treated weanling males. PCP caused a decrease in plasma corticosterone concentrations in 12-week-old female rats, but not in male rats or weanling females. PCP-treated male pups had significantly increased testis weight at 12 week of age. No overt signs of toxicity were noted throughout this study. Our results show that PCP exposure during development causes thyroid function vulnerability, testicular hypertrophy in adults, and aberrations of brain gene expression.

[Effects of pentachlorophenol on rat sertoli cells].
Yang S. Z., Han X. D., Chen W. and Yin D. Q.
Zhonghua Nan Ke Xue. 2005;11(4):261-3, 68.

OBJECTIVE: To detect the toxic effects of pentachlorophenol (PCP) on cultured rat Sertoli cells. METHODS: The viability of Sertoli cells was detected and morphological examination was performed, followed by flow cytometric assay to evaluate the toxic effect of PCP on rat Sertoli cells. RESULTS: MTT assay showed that PCP induced a concentration- and time-dependent decrease in Sertoli cell viability. Flow cytometric assay revealed that the number of dead Sertoli cells grew along with increased exposure to PCP. CONCLUSION: PCP, with obvious cytotoxic effects, can cause necrosis of Sertoli cells in vitro.

Flow cytometric assessment of changes in rat sperm mitochondrial function after treatment with pentachlorophenol.
Gravance C. G., Garner D. L., Miller M. G. and Berger T.
Toxicol In Vitro. 2003;17(3):253-7.

The fluorophore 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl carbocyanine iodide (JC-1) localizes to the mitochondria and is affected by membrane potential, fluorescing bright orange when the membrane potential is high and green when mitochondrial membrane potential is low. The present study used flow cytometric analysis of JC-1 staining patterns of large numbers of spermatozoa to detect chemical-induced alterations of sperm mitochondrial membrane potential. Cauda epididymal rat

spermatozoa were incubated with pentachlorophenol (PCP; 0.1 microM or 1.0 microM), a known uncoupler of mitochondrial oxidative phosphorylation. Microscopic evaluation showed that the midpiece (mitochondrial location) of live, highly motile spermatozoa stained bright orange, while the midpiece of live, non-motile spermatozoa stained green. The midpiece of slightly or non-progressively motile spermatozoa stained a faint orange-green. The percentage of spermatozoa stained bright orange and the total percentage of spermatozoa stained orange (bright orange+faint orange) in the control samples of spermatozoa were significantly higher ($P<0.001$) than in the 0.1 microM and 1.0 microM PCP treated samples. These data indicate that sperm mitochondrial membrane potential is highly sensitive to the uncoupling effects of PCP and that JC-1 staining and flow cytometric analysis may be a sensitive assay to detect the effect of toxicants on rat sperm mitochondrial function.

A study of the developmental toxicity potential of pentachlorophenol in the rat.
Bernard B. K. and Hoberman A. M.
Int J Toxicol. 2001;20(6):353-62.

Pentachlorophenol (penta, CAS #87-86-5) is primarily used as a wood preservative. As part of the USEPA pesticide reregistration process, the developmental toxicity (embryo-fetal toxicity and teratogenic potential) of commercially available penta was studied following oral gavage to presumed pregnant female Sprague-Dawley rats (Crl:CD BR VAF/Plus Subdivision F, 83-3). Both study design and penta purity met the requirements of the USEPA. Doses of 0 (corn oil), 10, 30, and 80 mg/kg/day were administered to the rats at concentrations of 0, 2, 6, and 16 mg/ml, respectively from day 6 to day 15 of presumed gestation. The dosage volume was 5 ml/kg, adjusted on each day of dosage based on individual body weights recorded immediately before intubation. The rats were sacrificed on day 20 of presumed gestation and necropsied. The number of corpora lutea in each ovary was recorded. The uterus was examined for pregnancy, number and distribution of implantations, early and late resorptions and live and dead fetuses. Each fetus was weighed, sexed, and examined for gross external, soft tissue and skeletal alterations. The no-observable-adverse-effect-level (NOAEL) for maternal toxicity in rats was determined to be 30 mg/kg/day of penta. The developmental NOAEL for penta in rats was also found to be 30 mg/kg/day. The lowest-observable-adverse-effect-level (LOAEL) for penta developmental toxicity (80 mg/kg/day) was associated with increased resorptions, reduced live litter size and fetal body weights, and caused increased malformations and variations. These NOAELs, derived using USEPA approved study designs, are higher than those previously reported using penta that is no longer commercially available in studies with non-approved experimental designs. Penta should not be identified as a selective

developmental toxicant in the rat because adverse effects on development of rat conceptuses occurred only at maternally toxic dosages.

Reproductive and endocrine function in rams exposed to the organochlorine pesticides lindane and pentachlorophenol from conception.
Beard A. P., Bartlewski P. M., Chandolia R. K., Honaramooz A. and Rawlings N. C.
J Reprod Fertil. 1999;115(2):303-14.

There is controversy over the potential endocrine modulating influence of pesticides, particularly during sensitive phases of development. In this study, ram lambs were exposed to lindane and pentachlorophenol from conception to necropsy at 28 weeks of age. The rams (and their mothers) were given untreated feed (n = 7) or feed treated with 1 mg kg-1 body weight per day of lindane (n = 12) or pentachlorophenol (n = 5). Semen was collected from 19 weeks onwards and reproductive behaviour was tested at 26 weeks. Serum was collected every 2 weeks and at 27 weeks every 15 min for 6 h during both day and night, and for 1 h before and 5 h after stimulation with GnRH, adrenocorticotrophic hormone and thyroid-stimulating hormone. The pesticides did not affect body weight and ejaculate characteristics, or cause overt toxicity. In pentachlorophenol-treated rams, scrotal circumference was increased. However, seminiferous tubule atrophy was more severe and epididymal sperm density was reduced in comparison with untreated rams at necropsy ($P < 0.05$). Thyroxine concentrations were lower in pentachlorophenol-treated rams than in untreated rams ($P < 0.05$). However, after thyroid-stimulating hormone treatment, the thyroxine response was unaltered. Reproductive behaviour was reduced in lindane-treated rams compared with control rams ($P < 0.05$). Serum LH and oestradiol concentrations during reproductive development, LH pulse frequency at 27 weeks and testosterone secretion after GnRH treatment were lower in lindane-treated rams than in untreated rams ($P < 0.05$). In summary, the effects of pentachlorophenol on the testis may be linked to a decrease in thyroxine concentrations, and reduced reproductive behaviour in lindane-treated rams may be related to decreased LH, oestradiol and testosterone concentrations.

Endocrine and reproductive function in ewes exposed to the organochlorine pesticides lindane or pentachlorophenol.
Beard A. P., Bartlewski P. M. and Rawlings N. C.
J Toxicol Environ Health Part A. 1999;56(1):23-46.

The effects of lindane (LIN, gamma-hexachlorocyclohexane) and pentachlorophenol (PCP) on reproduction and general endocrine function were examined in breeding ewes

as a model for wild and domestic ungulates, which may be exposed to low levels of pesticides that are potential endocrine-disrupting chemicals. Ewes (n = 13/group) were fed either a control untreated diet (CON), or a diet treated with LIN (1 mg/kg/d) or PCP (1 mg/kg/d) during the 5 wk prior to mating and throughout pregnancy and lactation. Mating response, ovulation rate, follicle and corpus luteum size, gestation length, pregnancy rate, lambing rate, and lamb birth weight were recorded. After weaning, 6 ewes from each group were bled at 15-min intervals for 8 h during the day and night and for 1 h before and 5 h after i.v. administration of gonadotropin-releasing hormone, thyroid-stimulating hormone (TSH), and adrenocorticotropin, to measure serum concentrations of luteinizing hormone, follicle-stimulating hormone, thyroxine (T4), and cortisol. Ewes were then killed and endocrine tissues examined histologically. Pregnancy rate as a result of matings taking place at the synchronized estrus was significantly decreased by the lindane treatment. However, PCP and lindane did not markedly affect any other aspect of reproductive function studied. In PCP-treated ewes, serum concentrations of T4 were significantly reduced compared to control ewes during the day and night; however, the T4 response to TSH was not altered by PCP treatment. No other measured endocrine parameters were consistently affected by lindane or PCP. Thyroid follicle size was significantly increased in the LIN and PCP ewes compared to the control ewes. Low serum concentrations of T4 in the PCP ewes may have resulted in increased TSH secretion and increased thyroid follicle size. In conclusion, although pesticide treatments had no serious adverse effects on reproductive function in breeding ewes, PCP reduced T4 concentration, which in the long term could influence reproductive and general performance.

Thyroid function and effects on reproduction in ewes exposed to the organochlorine pesticides lindane or pentachlorophenol (PCP) from conception.
Beard A. P. and Rawlings N. C.
J Toxicol Environ Health Part A. 1999;58(8):509-30.

There is concern over the potential endocrine-modulating effects of long-term exposure to pesticides. In this study, ewe lambs were exposed to lindane and pentachlorophenol (PCP) from conception to necropsy at 67 wk. of age. The ewe lambs (and their mothers) were given untreated feed (n = 6) or feed treated with 1 mg/kg body weight/day of lindane (n = 8) or PCP (n = 13). Estrus was synchronized at 32 wk. of age, and ewe lambs were exposed to vasectomized rams. Ewe lambs were then exposed to intact rams during the following two natural estrous periods and subsequent reproductive performance was monitored. Serum was collected every 2 wk. during development, daily during the synchronized cycle and frequently (every 15-60 min) for 6-18 h either with or without stimulation with thyroid-stimulating hormone (TSH) during the

synchronized luteal phase or TSH/thyroid-releasing hormone (TRH) at 65-66 wk of age. Ewe lambs fed a PCP-treated diet had a significantly reduced serum concentration of both T4 and free T4, and a reduction in the magnitude and duration of the T4 and free T4 response to TSH, despite normal endogenous levels of TSH and a normal TSH response to TRH. PCP exposure had a less detrimental influence on unstimulated T3 levels; however, the T3 (but not reverse T3) response to TSH was markedly reduced in PCP-treated ewe lambs. Ewe lambs given lindane also had a significantly reduced serum concentration of T4; however, despite continued exposure to lindane, T4 levels returned to normal by 10 wk. of age. Detrimental effects on reproductive function were only seen following estrous synchronization when both PCP and lindane exposure reduced the number of corpora lutea (CL) and total CL volume and increased luteinizing hormone (LH) pulse frequency. In addition, lindane-treated ewes had shorter estrous cycles and lower luteal progesterone concentrations. No marked effects of pesticides were seen on fertility following mating during natural estrous periods. In conclusion, the pesticides affected reproduction only after estrous synchronization, whereas PCP consistently disrupted thyroid function, most likely through a direct effect on the thyroid gland.

Effects of the pesticides carbofuran, chlorpyrifos, dimethoate, lindane, triallate, trifluralin, 2,4-D, and pentachlorophenol on the metabolic endocrine and reproductive endocrine system in ewes.
Rawlings N. C., Cook S. J. and Waldbillig D.
J Toxicol Environ Health Part A. 1998;54(1):21-36.

Many pesticides are used in the agricultural environment, and some may have the potential to disrupt reproductive or endocrine function. Ewes, in separate groups of 6, received orally into their rumen either empty gelatin capsules or capsules containing chlorpyrifos (12.5 mg/kg), trifluralin (17.5 mg/kg), lindane (2.5 mg/kg), or pentachlorophenol (2 mg/kg) 2 times per week for 43 d. Dimethoate (0.2 mg/kg), carbofuran (0.30 mg/kg), 2,4-dichlorophenoxyacetic acid (10 mg/kg), or triallate (5 mg/kg) was given 3 times per week. After 36 d of treatment, blood samples were taken every 12 min for 6 h for hormone analysis. Ewes were euthanized at the end of the study for necropsy and histopathology. No overt signs of toxicity were seen, and body weight was not affected by treatment. Carbofuran caused a significant increase in serum concentrations of thyroxine compared to control ewes, but all other pesticides, except trifluralin, resulted in a marked decrease in thyroxine concentrations. Serum concentrations of cortisol were significantly increased by trifluralin and chlorpyrifos. Concentrations of insulin in serum were markedly increased in ewes given dimethoate, lindane, trifluralin, triallate, and pentachlorophenol, and concentrations of estradiol were

also significantly increased in ewes given lindane and trifluralin. Mean serum concentrations of LH were markedly decreased by trifluralin, and basal LH concentrations were significantly decreased by lindane, dimethoate, and trifluralin but increased by triallate. Both pentachlorophenol and triallate caused a significant increase in severity of oviductal intraepithelial cysts in ewes. Data suggest that several currently used pesticides could influence serum concentrations of reproductive and metabolic hormones, particularly thyroxine, the major secretory product of the thyroid and a principal regulator of metabolism.

Reproductive efficiency in mink (Mustela vison) treated with the pesticides lindane, carbofuran and pentachlorophenol.
Beard A. P., McRae A. C. and Rawlings N. C.
J Reprod Fertil. 1997;111(1):21-8.

Mink are carnivores of agroforestry fringe habitats and are exposed to pesticides that biomagnify within the food chain. Some pesticides are thought to disrupt reproductive and endocrine functions. In Expt 1, four groups of mink (n = 10) were fed either a control diet, or diets treated with lindane (1 mg kg-1 day-1), carbofuran (0.05 mg kg-1 day-1) or pentachlorophenol (1 mg kg-1 day-1) from before breeding until weaning. Mink were mated twice, at 7-8 day intervals. The treatments had no effect on the proportion of mink accepting the first mating; however, lindane and pentachlorophenol caused a decrease in the percentage of females accepting the second mating. Lindane and pentachlorophenol caused a decrease in whelping rate, although litter size was not affected. Carbofuran had no effect on fertility. Mink that mated only once had a lower whelping rate than mink that mated twice; therefore, it could not be determined whether the decreased whelping rates were due to the lack of a second mating or to increased embryo loss. In Expt 2, two groups of mink (n = 15) were fed a control diet or a diet treated with lindane (1 mg kg-1 day-1) from before mating until weaning. Mink were mated twice on two consecutive days. Lindane did not affect mating response at either mating. Whelping rate, but not implantation rate, was decreased by the lindane treatment. The proportion of embryos lost after implantation (implantation scars not represented by kits at whelping) was increased by the lindane treatment. In conclusion, both lindane and pentachlorophenol decreased fertility in mink, and the lindane effect was primarily a result of embryo mortality after implantation.

Bovine spermatozoa as an in vitro model for studies on the cytotoxicity of chemicals: effects of chlorophenols.
Seibert H., Kolossa M. and Wassermann O.
Cell Biol Toxicol. 1989;5(3):315-30.

The suitability of ejaculated bovine spermatozoa as an in vitro model for the assessment of the cytotoxic potential of chemicals was evaluated using several endpoints: swimming activity, adenine nucleotide content, membrane integrity and oxygen consumption. A series of chlorophenols inhibited sperm motion (motility and velocity) in a concentration-dependent manner. This could be determined quantitatively and reproducibly by means of videomicrography and automatic computer image analysis. The sperm immobilizing potency increased with increasing chlorination and was positively correlated with lipophilicity. Concentrations which reduced the percentage of moving sperm to 50% of controls ranged from 43 microM for pentachlorophenol (PCP) to 1440 microM for 4-monochlorophenol (4-MCP). Determinations of adenine nucleotides and percentages of viable cells revealed qualitative differences between the action of PCP and the lower chlorinated phenols. While the latter decreased the total adenine nucleotide contents and the percentage of unstained cells in parallel to motion inhibition, no such changes occurred after exposure to immobilizing concentrations of PCP. Penta-, tetra- and trichlorinated phenols stimulated cellular respiration, indicating their uncoupling activity, at concentrations lower than those necessary for motion inhibition. The results indicate that bovine spermatozoa may become a useful in vitro model for the toxicological evaluation of chemicals providing quantitative as well as qualitative data.

Teratogenic potential of purified pentachlorophenol and pentachloroanisole in subchronically exposed Sprague-Dawley rats.
Welsh J. J., Collins T. F., Black T. N., Graham S. L. and O'Donnell M. W., Jr.
Food Chem Toxicol. 1987;25(2):163-72.

Male and female Sprague-Dawley (Spartan) rats were exposed to dietary levels of 0, 60, 200 or 600 ppm purified pentachlorophenol (PCP) or pentachloroanisole (PCA) for 181 days, through mating and pregnancy. The daily intakes of PCP were 0, 4, 13 or 43 mg/kg body weight and of PCA were 0, 4, 12 or 41 mg/kg body weight. Animals exposed to PCP generally consumed more food than control animals during pregnancy. Dams at the high-dose level of both compounds showed evidence of toxicity, weighing less on day 0 of gestation and gaining less throughout pregnancy than did the controls.

Dams exposed to the high dose of PCP gained less weight during pregnancy (exclusive of the gravid uterus) than control dams. At the 43 mg/kg/day dose level PCP was embryolethal. Foetuses at the lower dose levels of PCP exhibited dose-related decreases in body weights. A reduction in crown-rump length and an increase in foetal skeletal variations were seen at 13 mg/kg/day in PCP animals only. An intake of 41 mg PCA/kg/day was associated with a decrease in the number of corpora lutea and in embryolethality. PCA exposure also resulted in reductions in foetal body weight and crown-rump lengths of males at 4 and 41 mg/kg/day. Female foetuses were unaffected.

Effects of transplacental exposure to chlorinated phenols.
Exon J. H. and Koller L. D.
Environ Health Perspect. 1982;46:137-40.

Female rats were exposed to 0, 5, 50 or 500 ppm of 2-chlorophenol (2-CP) or pentachlorophenol (PCP). The study was designed to produce progeny which were exposed to the chlorophenolic compounds both prenatally and postnatally. Percent conception, litter size, birth weight, and number of stillbirths was determined at parturition. Hematologic parameters and body weights of the progeny were recorded at weaning age (3 weeks). Effects on reproduction were observed in both the 2-CP and PCP-exposed groups, as indicated by decreased litter sizes and increased number of stillborn. The data indicate that these chlorinated phenolic compounds may be feto- or embryotoxic at high doses. Effects on hematologic parameters were not observed. Further study involving transplacental and chronic exposures to these chlorophenolic compounds appears warranted.

Placental transfer and teratology of pentachlorophenol in rats.
Larsen R. V., Born G. S., Kessler W. V., Shaw S. M. and Van S. D. C.
Environ Lett. 1975;10(2):121-8.

Pentachloro[U-14C]phenol was administered orally to Charles River CD strain pregnant rats on day 15 of gestation. Concentrations found in the placentas and fetuses up to 32 hr remained very small indicating that the amount that passes through the placental barrier is negligible. Unlabeled compound was administered on days 8, 9, 10, 11, 12, and 13 of gestation. The incidence of resorptions in the treated animals was not significantly greater than that in the controls. Although malformations were observed, the number was minimal and could have been due to the toxic effects of the compound on the maternal rat.

Oral (gavage) two-generation (one litter per generation) reproduction study of pentachlorophenol (penta) in rats.
Bernard B. K., Hoberman A. M., Brown W. R., Ranpuria A. K. and Christian M. S.
Int J Toxicol. 2002;21(4):301-18.

The potential for pentachlorophenol (penta) to induce general and reproductive/developmental toxicity was evaluated in Crl Sprague-Dawley rats, employing a two-generation reproduction toxicity study. Penta was administered by gavage at doses of 0, 10, 30, and 60 mg/kg/day. In both generations, the parental animals (30/sex/group) were intubated daily for 10 weeks before cohabitation and continuing through cohabitation, gestation, and lactation periods. Intubation of the F1 generation was begun 28 days postpartum. Animals were evaluated daily for mortality and general toxicity (clinical observations, body weights and gains, feed consumption). Organ weights were recorded and histopathological evaluations were made. Specific indices of reproductive function evaluated included estrous cycles, mating and fertility, parturition, lactation, viability, and growth and development of offspring, including sexual maturation, sperm parameters, and numbers of ovarian primordial follicles. All deaths in the parental rats were unrelated to penta. Expected metabolic effects of penta, sporadic increased liver weights associated with hepatocellular centrilobular hypertrophy and vacuolation and lipofuscin pigmentation, were evident in the 10-, 30-, and 60-mg/kg/day dose group P1 and F1 animals. Toxicity, in the form of liver pathology (single cell necrosis), reduced body weights and associated reductions in organ weights, and reduced feed consumption were noted in both generations at the 30- and 60-mg/kg/day doses. Developmental toxicity associated with these doses included reduced pup weights and viability. The 60-mg/kg/day dose also resulted in delayed sexual maturation, decreased spermatid counts, small prostates and testes, decreased implantations, reduced fertility, and increased resorptions of embryos. Based on these results, it was concluded that 30 mg/kg/day is the lowest-observable-adverse-effect level (LOAEL) and 10 mg/kg/day is the no-observable-adverse-effect level (NOAEL) for both reproductive and general toxicity. These findings are consistent with results from previously conducted studies wherein reproductive/developmental toxicity was observed only at doses that also induced general toxicity. It differs from previous findings in that the NOAEL for general toxicity is two to three times higher for the more pure product than for products produced and tested previously. In addition, the results did not indicate bioaccumulation of penta. Thus, penta did not selectively affect reproduction or development of the offspring of rats at a dose of 10 mg/kg/day, a dose that is 7000 to 20,000 times higher than human exposure.

Reproductive effects in mink (Mustela vison) exposed to the pesticides Lindane, Carbofuran and Pentachlorophenol in a multigeneration study.
Beard A. P. and Rawlings N. C.
J Reprod Fertil. 1998;113(1):95-104.

The mammalian reproductive system is sensitive to exposure to endocrine disrupting chemicals, particularly during sexual maturation. The purpose of this study was to examine reproductive function in second and third generation male and female mink exposed to pesticides from conception to maturity. The mink were fed untreated feed or feed treated with Lindane (1 mg kg-1 day-1), Carbofuran (0.05 mg kg-1 day-1) or Pentachlorophenol (1 mg kg-1 day-1) from the time they were weaned. The second generation mink had also been exposed to the pesticides in utero and from their mother's milk as their mothers were similarly fed pesticides, from 3 weeks before breeding. The third generation mink were the offspring of mink (second generation females) who had themselves undergone long-term exposure to pesticides from conception onwards. Blood samples and endocrine tissues were obtained at necropsy from both generations of mink. No overt signs of toxicity were seen. The pesticides did not affect the percentage of mink mated. Lindane treatment reduced the proportion of mated mink that subsequently whelped ($P < 0.1$) and the litter size of mink that whelped ($P < 0.05$). Testis size was reduced in the Lindane-treated, third generation males ($P < 0.05$). Serum concentrations of cortisol, testosterone and oestradiol were not affected by any pesticide treatment; however, thyroxine concentration was reduced by Pentachlorophenol ($P < 0.05$). In conclusion, exposure of mink to Lindane from conception resulted in a decrease in reproductive efficiency when they were subsequently mated, leading to a 60% reduction in the number of kits born.

Pentachlorophenol exposure causes Warburg-like effects in zebrafish embryos at gastrulation stage.
Xu T., Zhao J., Hu P., Dong Z., Li J., Zhang H., Yin D. and Zhao Q.
Toxicol Appl Pharmacol. 2014;277(2):183-91.

Pentachlorophenol (PCP) is a prevalent pollutant in the environment and has been demonstrated to be a serious toxicant to humans and animals. However, little is known regarding the molecular mechanism underlying its toxic effects on vertebrate early development. To explore the impacts and underlying mechanisms of PCP on early development, zebrafish (Danio rerio) embryos were exposed to PCP at concentrations of 0, 20 and 50 mug/L, and microscopic observation and cDNA microarray analysis were subsequently conducted at gastrulation stage. The morphological observations

revealed that PCP caused a developmental delay of zebrafish embryos in a concentration-dependent manner. Transcriptomic data showed that 50 mug/L PCP treatment resulted in significant changes in gene expression level, and the genes involved in energy metabolism and cell behavior were identified based on gene functional enrichment analysis. The energy production of embryos was influenced by PCP via the activation of glycolysis along with the inhibition of oxidative phosphorylation (OXPHOS). The results suggested that PCP acts as an inhibitor of OXPHOS at 8 hpf (hours postfertilization). Consistent with the activated glycolysis, the cell cycle activity of PCP-treated embryos was higher than the controls. These characteristics are similar to the Warburg effect, which occurs in human tumors. The microinjection of exogenous ATP confirmed that an additional energy supply could rescue PCP-treated embryos from the developmental delay due to the energy deficit. Taken together, our results demonstrated that PCP causes a Warburg-like effect on zebrafish embryos during gastrulation, and the affected embryos had the phenotype of developmental delay.

Individual and joint toxic effects of pentachlorophenol and bisphenol A on the development of zebrafish (Danio rerio) embryo.
Duan Z., Zhu L., Zhu L., Kun Y. and Zhu X.
Ecotoxicol Environ Saf. 2008;71(3):774-80.

Investigation of the toxicological effects of pentachlorophenol (PCP) and bisphenol A (BPA) alone and in combination was carried out following the method of the early life stage (ELS) test on zebrafish embryos. Both chemicals revealed lethal and sub-lethal effects, such as no blood flow, cardiac edema, delayed hatching, and tail malformations. According to their median effective concentrations (EC(50) values) in the single exposure, the toxic level of PCP was about two orders of magnitude higher than that of BPA. Result of the joint action modes varied depending on different endpoints. Synergistic action was observed based on the endpoint of 24h mortality and antagonistic effect displayed based on the endpoint of 72 h cardiac edema. It was also found that the toxicity of PCP would be enhanced with the addition of BPA even below its no observed effect concentration (NOEC) level at the endpoint of 32 h with no blood flow, and the level of the increase was influenced by the toxic unit (TU) ratio.

[Toxicity effects of pentachlorophenol on Brachydanio rerio].
Zheng M. and Zhu L.
Ying Yong Sheng Tai Xue Bao. 2005;16(10):1967-71.

With embryo development technique, this paper determined the toxicity of pentachlorophenol (PCP) to Brachydanio rerio embryos. The results showed that the

special effect duration of PCP on embryos was within 6 hours after embryos incubation. PCP could markedly inhibit the development of Brachydanio rerio embryos, and cause its malformation and even death. There were different toxicity endpoints which could be observed in embryos exposed to PCP with different time. The lethal effect sensitivity of embryos incubated after 48 hours was getting lower when the exposure time was getting shorter, the LC50 being the minimum (70.8 microg x L(-1)) for 0 hpf embryos, and the maximum (831.8 microg x L(-1)) for 24 hpf embryos. The acute toxicological endpoint of Brachydanio rerio embryos was in order of edema, no blood-circulation and heartbeat > incubation rate > stopping growth, and the most sensitive endpoints of Brachydanio rerio embryos to pentachlorophenol were no blood-circulation and half lethal concentration at 48 hrs.

B. Meeting abstracts reporting developmental or reproductive toxicity

Pentachlorophenol: Comparison of Animal and Human Reproductive and Developmental Toxicity.
Davis J. A., Jr., Laessig S. A. and Kimmel C. A.
Birth Defects Res A Clin Mol Teratol. 2006;76(5):385.

Animal toxicity data are generally considered to be predictive of human developmental toxicity, but quantitative comparisons have been limited to a few drugs and other chemicals due to a lack of adequate human data. Pentachlorophenol (PCP) is a chlorinated pesticide for which adequate human and animal data were available to perform a quantitative comparison of reproductive and developmental toxicity data. PCP is used primarily ,in the treatment of railroad ties, utility poles, and fence posts and was one of the most widely used wood preservatives in the United States before its restriction in 1984. The general public is chronically exposed to low levels of PCP, but the route(s) of exposure are unknown. Developmental studies identified and analyzed in this study included 12, human, 7 rat, and 1 rabbit study. The published data on PCP developmental toxicity in humans and animals were first extracted into a database and evaluated for reliability and the likelihood of causation. Reproductive and developmental outcomes in animal models and humans were then compared in order to assess the predictability of animal studies for human outcomes. The most predictive outcomes for human developmental toxicity were resorptions and altered birth and fetal weight in rats, which occurred at doses lever than those resulting in maternal toxicity or effects on fertility. Species-specific differences in sensitivity were observed. Humans were more sensitive to PCP exposure than rats, exhibiting adverse effects at dose levels approximately one order of magnitude lower, while .rabbits were the .least sensitive. Relative differences .In the sensitivity .of rats and rabbits to gestational PCP exposure

potentially resulted from differences in the timing, dose levels, and pattern of exposures. The comparison of animal and; human developmental toxicity data was 1imited by differences in dosing and exposure ascertainment, PCP formulations used, timing of exposure, and lack of adequate or complete data for some outcomes.

C. Studies reporting no developmental or reproductive toxicity

1H NMR to investigate metabolism and energy supply in rhesus macaque sperm.
Lin C. Y., Hung P. H., VandeVoort C. A. and Miller M. G.
Reprod toxicol. 2009;28(1):75-80.

Sperm ATP is derived primarily from either glycolysis or mitochondrial oxidative phosphorylation. In the present studies, (1)H NMR spectroscopy was used to characterize the metabolite profile in primate sperm treated either with alpha-chlorohydrin (ACH), a known inhibitor of sperm glycolysis or pentachlorophenol (PCP), an uncoupler of oxidative phosphorylation. Sperm were collected from monkeys in the fall and spring, washed and incubated with either the media control, ACH (0.5mM) or PCP (50 microM). Using principal components analysis, PC1 scores plot indicated that the greatest level of variance was found between fall and spring samples and not chemical-treated samples. However, PC4 scores plot did show a consistent effect of ACH treatment. From the PC1 loadings plot, metabolites contributing to the seasonal differences were higher levels of formate in the fall and higher levels of carnitine and acetylcarnitine in the spring as well as possible differences in lipoprotein content. The PC4 loadings plot indicated that ACH treatment decreased lactate and ATP consistent with inhibition of glycolysis. Carnitine also was decreased and acetylcarnitine increased although the latter was not statistically significant. With PCP-treated sperm, no difference between control and treated samples could be discerned suggesting either that primate sperm are insensitive to uncoupling agents or that glycolysis played the more important role in maintaining sperm ATP levels. Overall, NMR studies may prove useful in the development of metabolomic markers that signal sperm metabolic impairments and have the potential to provide useful biomarkers for reproductive health.

Developmental toxicity study of pentachlorophenol in the rabbit.
Bernard B. K., Ranpuria A. K. and Hoberman A. M.
Int J Toxicol. 2001;20(6):345-52.

The potential for developmental toxicity of pentachlorophenol (penta) was studied in New Zealand white rabbits at doses of 0 (corn oil), 7.5, 15, and 30 mg/kg/day

administered by gavage on days 6 to 18 of gestation. The rabbits were sacrificed on day 29 of presumed gestation and necropsied. Measurements included number of corpora lutea, pregnancy, number and distribution of implantations, early and late resorptions, live and dead fetuses, fetal weight, gender, and gross external, soft tissue, and skeletal alterations. The mid and high doses reduced maternal body weight gain; the high dose caused transient weight loss and reduced feed consumption. There were no effects on embryofetal development at any of the doses evaluated. Based on these data, the maternal no-observable-adverse-effect level (NOAEL) is 7.5 mg/kg/day, while the developmental NOAEL is 30 mg/kg/day. Penta is not a developmental toxicant in a nonrodent animal model.

An assessment of the potential testicular toxicity of 10 pesticides using the mouse-sperm morphology assay.
Osterloh J., Letz G., Pond S. and Becker C.
Mutat Res. 1983;116(3-4):407-15.

Dinitrobutylphenol, chlorbenzilate, atrazine, Ordram, Telone (dichloropropene), pentachlorophenol (technical and reagent grades), Benomyl, DBCP (dibromochloropropane), and carbaryl were tested over a range of 7 doses in the mouse to assess their testicular toxicity. Measures of potential toxicity were sperm morphology, sperm counts and testicular weights. Each pesticide was injected intra-peritoneally in a single dose on each of 5 days. Testicular toxicity was assessed at 35 days. None of the pesticides tested, including the known human male testicular toxin, DBCP, produced statistically significant differences in the parameters from vehicle-injected controls.

D. Related articles

Effects of multigenerational exposures of D. magna to environmentally relevant concentrations of pentachlorophenol.
Chen Y., Huang J., Xing L., Liu H., Giesy J. P., Yu H. and Zhang X.
Environ Sci Pollut Res Int. 2014;21(1):234-43.

The re-emergence of schistosomiasis has given rise to ubiquitous concentrations of the primary control agent pentachlorophenol (PCP) in the environment, especially in the surface waters of China. In this study, the effects of environmentally relevant concentrations of PCP, namely, 0.0002, 0.002, 0.02, 0.2, and 2 μmol/L on survival, age at first reproduction, fecundity, length of mothers, and number of molts of Daphnia magna were studied over three generations. The survival of D. magna exposed to 2

mumol/L was significantly affected in the three generations. Toxic effects were enhanced in later generations. Age at first reproduction of F1 and F2 D. magna was significantly slower than that of the controls. The total number of offspring per female exposed to concentrations of 0.002 mumol/L or greater was less (23.5 to 67.6, 9.4 to 73.7, and 3.6 to 83.7%) than that of the controls in the F0, F1, and F2 generations, respectively. The body length of mothers significantly decreased (4.7 to 6.8, 9.6 to 15.1, and 13.3 to 23.2%) after exposure to 0.002 mumol/L or greater than those of unexposed individuals in the F0, F1, and F2 generations, respectively. Dose-response relationships between concentrations of PCP and length and number of molts of D. magna were observed in the F0 to F2 generations. PCP concentrations on the surface waters of China caused adverse effects to D. magna, which increased over successive generations. Significant effects were observed in the third generation. The multigenerational studies were more sensitive than the single-generation experiments. Thus, multigenerational exposure may be more predictive of chronic exposure under field conditions.

Toxic effects of pentachlorophenol, azinphos-methyl and chlorpyrifos on the development of Paracentrotus lividus embryos.
Buono S., Manzo S., Maria G. and Sansone G.
Ecotoxicology. 2012;21(3):688-97.

The application of many current-use pesticides has increased after the disuse of persistent, bioaccumulative or toxic ones as DDT or chlordane. Many of the used pesticides are considered less dangerous towards the environment for their physico-chemical properties. This study investigated the toxic effects of three current-use pesticides, pentachlorophenol (PCP), azinphos-methyl (AZM), and chlorpyrifos, on Mediterranean sea urchin Paracentrotus lividus early development and offspring quality. The experimental results showed that the most toxic pesticides were PCP and AZM at EC50 level. Nevertheless at low concentration PCP resulted the less toxic compound and showed EC1 value more protective than NOEC. PCP at high concentration seemed to modify cytoskeleton assembly, while at low concentrations, it could alter the deposition of the larval skeleton. OPs at low concentrations until 300 mug/l showed a similar toxicological behaviour with a trend corresponding to the pesticide concentrations. At high concentration (500 mug/l) the effect mainly observed was the embryos pre-larval arrest. This investigation highlighted the relevance to evaluate, in coastal seawaters, the levels of the used pesticides to understand the real impact on benthic populations mainly in sites characterized by intensive agriculture or floriculture activities, such as the coastal areas of the Mediterranean Sea.

Endocrine disrupting effects of herbicides and pentachlorophenol: in vitro and in vivo evidence.
Orton F., Lutz I., Kloas W. and Routledge E. J.
Environ Sci Technol. 2009;43(6):2144-50.

The potential for agricultural chemicals to cause endocrine disruption (ED) in humans and wildlife is an increasing concern; however, the effects of commonly used pesticides at environmentally relevant concentrations are largely unknown. Therefore, 12 environmentally relevant pesticides (11 herbicides and pentachlorophenol (PCP)) were tested for their endocrine disrupting potential in two in vitro assays. A recombinant yeast screen was used to detect receptor mediated (anti-) estrogenic and (anti-) androgenic activity (concentration range: 0.01-1000 microM), and cultured Xenopus oocytes were used to measure effects on the ovulatory response and ovarian steroidogenesis (concentration range: 0.00625-62.5 microM). Eleven pesticides were active in at least one assay (isoproturon, diuron, linuron, 4-chloro-2-methylphenoxy acetic acid (MCPA), mecoprop, atrazine, simazine, PCP, trifluralin, chlorpropham, bentazone), and one had no effect (2,4-dichlorophenoxy acetic acid (2,4,-D)). The most common effects were antiestrogenic/ antiandrogenic activity in the yeast screen, and inhibition of ovulation in vitro, accompanied by decreased testosterone production. Estrogenic activity was never observed. In addition, the most potent compound identified in vitro (PCP) was tested for ED activity in vivo. A short-term exposure (6 days) of adult female Xenopus to low concentrations (0.1 or 1 microg/L; 0.375 or 3.75 nM) resulted in minor alterations in plasma hormone levels and toxic effects on the ovary. Changes in in vitro human chorionic gonadotropin (hCG) stimulated hormone production in ovarian follicles from exposed individuals was also observed. In conclusion, novel effects of herbicides and PCP at environmentally relevant concentrations were found, and the effects of these compounds on humans and/or wildlife warrant further investigation.

[Exploration of developmental toxicity mechanism of pentachlorophenol using cDNA microarray].
Wu Z. Y., Hu P., Zhao Q. S. and Yin D. Q.
Huan Jing Ke Xue. 2009;30(11):3382-7.

0hpf zebrafish embryos were exposed to 50 microg/L pentachlorophenol(PCP) for 8h in vitro. Total RNA sample was extracted then and hybridized with Affymetrix Zebrafish Genome Array representing approximately 14 900 transcripts. A total of 1 149 transcripts was significantly up-regulated while 501 transcripts were down-regulated. Bioinformatic tools were used for further analysis. The result indicated that genes with significant expression changes were related to molecular functions including antioxidant

activity, signal transducer activity, translation regulator activity, transcription regulator activity, et al. Genes regulated by BMP signals, FGF signals, and Nodal signals including smad2, smad5, bmp4, bmp7, flh, n-ras may involve in the developmental toxicity of PCP, with Signal log ratios of 4.6, 2.1, 1.6, 1.0, 1, 1.3, 1.0, respectively. This investigation may provide new biomarkers to further study of the developmental toxicity of PCP.

Difference in the sensitivity to chemical compounds between female and male neonates of Daphnia magna.
Ikuno E., Matsumoto T., Okubo T., Itoi S. and Sugita H.
Environ Toxicol. 2008;23(5):570-5.

Daphnia magna usually produce female offspring by parthenogenesis, and thus only female neonates are used to evaluate the environmental toxicity to chemicals. Additionally, it is known that male daphnids are induced by exposure to a juvenile hormone, methyl farnesoate, during late ovarian development. In this study, we investigated the concentration of methyl farnesoate in a 24-h exposure producing 100% males, and the difference in sensitivity to chemical compounds, potassium dichromate, pentachlorophenol, and paraquat, between females and males, referring to OECD Test Guideline 202. The results show that the minimum concentration for 100%-male induction of methyl farnesoate in adult females was 50 microg/L. In addition, acute toxicity tests (immobility test) with the other chemicals showed that male neonates have higher tolerance to potassium dichromate and pentachlorophenol than females for at least 24 h after birth, while no sex difference was observed in the sensitivity to paraquat. The differences in the median effective concentrations in these compounds between female and male neonates suggest two different overall modes of action. Using female daphnids for environmentally toxicity testing seems reasonable, since the females are more sensitive to chemicals than males. Furthermore, the method of male induction established in this study could be used for screening of endocrine disruptors.

Sonophotocatalysis of endocrine-disrupting chemicals.
Tokumoto T., Ishikawa K., Furusawa T., Ii S., Hachisuka K., Tokumoto M., Tsai H. J., Uchida S. and Maezawa A.
Mar Environ Res. 2008;66(3):372-7.

Sonolysis and photolysis often exhibit synergistic effects in the degradation of organic molecules. An assay of fish oocyte maturation provides an appropriate experimental system to investigate the hormonal activities of chemical agents. Oocyte maturation in fish is triggered by maturation-inducing hormone (MIH), which acts on receptors on the

oocyte surface. A synthetic estrogen, diethylstilbestrol (DES), possesses inducing activity of fish oocyte maturation, and a widely used biocide, pentachlorophenol (PCP), exhibits a potent inhibitory effect on fish oocyte maturation. In this study, the effects of the combined treatment by sonolysis with photolysis (sonophotocatalysis) to diminish the hormonal activity of DES and the maturation preventing activity of PCP was examined. By sonophotocatalysis, hormonal activity of DES was completely lost within 30 min and the inhibiting activity of PCP was lost within 120 min. These results demonstrated that sonophotocatalysis is effective for diminishing the endocrine-disrupting activity of chemical agents.

Effects of pentachlorophenol on Galba pervia, Tubifex sinicus and Chironomus plumousus larvae.
Song Z. H.
Bull Environ Contam Toxicol. 2007;79(3):278-82.

The 24-h median lethal concentrations of pentachlorophenol to Chironomus plumousus, Tubifex sinicus and Galba pervia were 0.302, 0.977 and 0.293 mg/L, respectively. Bioconcentration factors of C. plumousus, T. sinicus and G. pervia to pentachlorophenol were 108, 367 and 85 at 0.02 mg/L pentachlorophenol, respectively. As pentachlorophenol concentration increased, the G. pervia egg hatching rates became lower, and the total hatched time became longer. Pentachlorophenol teratogenesis was demonstrated by observing the deformation of C. plumousus larvae mentum.

The relative sensitivity of growth and reproduction in the springtail, Folsomia candida, exposed to xenobiotics in the laboratory: an indicator of soil toxicity.
Crouau Y. and Moia C.
Ecotoxicol Environ Saf. 2006;64(2):115-21.

The Folsomia candida reproduction test [ISO, 1998. Soil quality--Inhibition of reproduction of Collembola (Folsomia candida) by soil pollutants. International Standard Organization Report 11267, 1998, Geneva] is used to evaluate the ecotoxicological risks of contaminants in soils. The aim of this study was to compare the sensitivity of growth and reproduction of F. candida to four xenobiotics: two metals (Cd, Al), one metalloid (As), and one organic compound (pentachlorophenol). We showed that reproduction is a slightly more sensitive parameter than growth: EC(20) for reproduction was 1.25 microg/g dry soil for arsenic, 56 microg/g for cadmium, 97.5 microg/g for aluminum, and 41.7 microg/g for pentachlorophenol. The corresponding EC(20) values for growth were 2.8, 65, 630, and 94.6 microg/g. Keeping in mind that a growth test needs fewer juveniles and less time than a reproduction test, we conclude that the two

parameters are complementary and could be used for a better ecotoxicological evaluation of contaminants. However, the relative growth and reproduction sensitivities should be tested with more chemicals before growth could be considered as a good alternative for a faster sublethal test.

Effects of pentachlorophenol on the reproduction of Japanese medaka (Oryzias latipes).
Zha J., Wang Z. and Schlenk D.
Chem Biol Interact. 2006;161(1):26-36.

Pentachlorophenol (PCP) is widely used to control termites and protect wood from fungal-rot and wood-boring insects, and is often detected in the aquatic environment. Few studies have evaluated PCP as an environmental endocrine disruptor. In the present work, Japanese medaka (Oryzias latipes) was exposed to PCP for 28 days (F0 generation) with subsequent measurements of vitellogenin (VTG), hepatic 7-ethoxyresorufin-O-deethylase (EROD), and reproductive endpoints. Plasma VTG significantly increased in male fish treated with PCP concentrations lower than 200 microg/l and decreased in male and female animals exposed to 200 microg/l. Hepatic EROD from female fish increased when PCP exposure concentrations exceeded 20 microg/l, but decreased in the 200 microg/l PCP treatment group. Fecundity and mean fertility of female medaka decreased significantly in the second and third week following exposure concentrations greater than 100 microg/l, and testis-ova of male medaka was observed at PCP concentrations greater than 50 microg/l. Histological lesions of liver and kidney occurred when exposure concentrations exceeded 50 microg/l. In F1 generations, the hatching rates and time to hatch of offspring were significantly affected in fish exposed to 200 microg/l. These results indicated that PCP exposure caused responses consistent with estrogen and aryl hydrocarbon receptor activation as well as reproductive impairment at environmentally relevant concentrations.

Induction and inhibition of oocyte maturation by EDCs in zebrafish.
Tokumoto T., Tokumoto M. and Nagahama Y.
Reprod Biol Endocrinol. 2005;3:69.

BACKGROUND: Oocyte maturation in lower vertebrates is triggered by maturation-inducing hormone (MIH), which acts on unidentified receptors on the oocyte surface and induces the activation of maturation-promoting factor (MPF) in the oocyte cytoplasm. We previously described the induction of oocyte maturation in fish by an endocrine-disrupting chemical (EDC), diethylstilbestrol (DES), a nonsteroidal estrogen. METHODS: In this study, stimulatory and inhibitory effects of EDCs and natural steroids

on oocyte maturation were examined in zebrafish. For effective agents, some details about the mechanism in induction or inhibition of maturation were examined. Possible groups of DES interacting with the MIH receptor are discussed based on relative potency of steroids to induce maturation. RESULTS: Among agents tested, tamoxifen (TAM) and its metabolite 4-hydroxytamoxifen (4-OHT) showed stimulatory activity similar to DES. The time courses of the change in germinal vesicle breakdown and an intracellular molecular event (the synthesis of cyclin B) induced by TAM were indistinguishable from those induced by MIH. In contrast, pentachlorophenol (PCP) had a potent inhibitory effect on MIH-induced oocyte maturation. PCP inhibited not only MIH-induced maturation but also DES- and TAM-induced maturation. Methoxychlor also inhibited maturation when oocytes were pre-treated with this agent. CONCLUSION: These results suggest that EDCs act as agonists or antagonists in the induction of oocyte maturation in fish.

Sertoli cell junctional proteins as early targets for different classes of reproductive toxicants.
Fiorini C., Tilloy-Ellul A., Chevalier S., Charuel C. and Pointis G.
Reprod Toxicol. 2004;18(3):413-21.

In the testis, Sertoli cells establish intercellular junctions that are essential for spermatogenesis. The SerW3 Sertoli cell line displays some features of native Sertoli cells. Western blot and immunofluorescence analyses showed that SerW3 Sertoli cells expressed typical components of tight (occludin and zonula occludens-1), anchoring (N-cadherin) and gap (connexin 43) junctions. Testicular toxicants (DDT, pentachlorophenol, dieldrin, dinitrobenzene, cadmium chloride, cisplatin, gossypol, bisphenol A and tert-octylphenol) affected intercellular junctions by either reducing the amount or inducing aberrant intracellular localization of these membranous proteins. Phosphodiesterase inhibitors (isobutyl methylxantine, rolipram, zaprinast, zardaverine) did not alter junctional-complex component levels but caused a rapid and reversible redistribution of these proteins to the cytoplasmic compartment. The present study showed that occludin, ZO-1, N-cadherin and specifically Cx43 could be early targets for testicular toxicants. The SerW3 cell line therefore appears as a useful in vitro model to evaluate molecules with potential anti-reproductive effects.

Pentachlorophenol (PCP) bioaccumulation and effect on heat production on salmon eggs at different stages of development.
Maenpaa K. A., Penttinen O. P. and Kukkonen J. V.
Aquat Toxicol. 2004;68(1):75-85.

In this study, pentachlorophenol (PCP) bioaccumulation and its effect on heat dissipation was studied in eggs of the lake salmon (Salmo salar m. sebago). In bioaccumulation studies, the eggs were exposed to low concentrations (0.051-0.056 micromol/l, 13.583-14.915) of waterborne [14C]-labeled PCP at two developmental stages: (1) 3 weeks after fertilization, and (2) just before hatching. The effect of PCP on egg heat dissipation was measured by a microcalorimeter after exposing the eggs to gradual concentrations (0-0.992 micromol/l) of PCP for 48 h. After both the bioaccumulation and heat dissipation experiments, the eggs were dissected and the concentrations of PCP in tissue were determined separately for eggshell, yolk and embryo. The bioaccumulation studies showed that PCP accumulates more in the eggs at the late developmental stage. Bioconcentration factors (BCF) for different tissues were 3-42 times higher for the eggs at the late developmental stage compared with the eggs that were incubated only for 3 weeks. In early developmental stage, the eggshell adsorbs a large portion of the chemical. In late developmental stage, the actual embryo accumulated both proportionately and totally more than other dissected tissues in the beginning of the exposure, but eventually the yolk accumulated highest total amount of the chemical. A probable reason for the higher PCP body burden in the late developmental stage is that the respiration rate and metabolic activity of the embryo increases as it grows. The salmon eggs responded to an exposure to PCP with an elevated rate of heat dissipation. The threshold concentration above which the embryo heat dissipation was amplified was 29.64 micromol/kg embryo wet weight (ww) or 0.28 micromol/l. The highest embryo heat production was measured at the exposure concentration of 0.992 micromol/l. At higher exposure concentrations the heat dissipation decreased. The basic findings of the study are that PCP accumulates in growing embryonic tissue and is able to change the physiology of developing embryo.

Modifications of the topical Japanese medaka (Oryzias latipes) embryo larval assay for assessing developmental toxicity of pentachlorophenol and p, p'-dichlorodiphenyltrichloroethane.
Owens K. D. and Baer K. N.
Ecotoxicol Environ Saf. 2000;47(1):87-95.

One method currently available for investigating developmental toxicity in teleost species is the Japanese medaka embryo larval assay (MELA). In the present study, the MELA was modified to evaluate repeated topical exposures to pentachlorophenol (PCP) and p, p'-dichlorodiphenyltrichloroethane (DDT) and to identify sensitive stages of embryonic development. A single topical exposure using embryos at 48 h postfertilization resulted in a statistically significant increase in embryo mortality at 688 and 1250 ng PCP/egg compared with controls. In contrast, the toxicity following exposure to 11, 36, 78, 120, 208, and 400 ng DDT/egg was expressed only in larvae after hatching. Results further demonstrate that the MELA can be optimized to accommodate repeated daily topical exposures starting at 48 h postfertilization and ending at 120 h postfertilization. In addition, the neurula stage (24 h postfertilization) represented the most sensitive embryonic stage following a single topical exposure of PCP. However, no differences were observed in the sensitivity of embryonic stages following DDT exposure. The modified MELA was also used to evaluate sediment extracts contaminated with DDT metabolites obtained from the Tensas River, Louisiana. Results indicate that there is a low potential for developmental toxicity using the present extraction and exposure scenario even though elevated levels of DDE and toxaphene currently exist in several adult fish species at this site. The MELA as a screen for evaluating the potential for developmental toxicity of contaminated sediments is discussed.

Toxic responses of Japanese medaka (Oryzias latipes) eggs following topical and immersion exposures to pentachlorophenol.
Helmstetter M. F. and Alden R. W. d.
Aquat Toxicol. 1995;32(1):15-29.

Oryzias latipes eggs were exposed to a wide range of concentrations of pentachlorophenol (PCP) using the medaka embryo-larval assay (MELA) topical protocol as well as a standard aquatic immersion protocol. The immersion concentrations were developed as equivalents to the topical doses using a bioconcentration factor (BCF) of 770 for PCP. The lethal and sublethal results were compared in relation to salinity (0 and 20 ppt), as well as between the two treatment procedures. The results indicated that the effect of salinity on PCP toxicity in medaka

eggs is minimal, with 21-day ED50 values of 0.0558 ug PCP/egg and 0.0618 ug PCP/egg for the freshwater and 20 ppt exposures, respectively. In addition, similar sublethal responses were observed in the two treatment types. These results indicate that there appears to be little stress of salinity itself on the PCP toxicity to medaka eggs. Further, using a published BCF for PCP, it was possible to develop immersion concentrations which resulted in similar responses to those observed for the topical MELA treatment. This confirmatory finding suggests that, given the proper information (e.g., a BCF), the MELA topical exposure can be used for chemicals for which an immersion exposure regime may prove problematic. Conversely, the combination of topical and immersion toxicity data may allow BCFs to be estimated for embryonic stages for which direct bioaccumulation measurements cannot be made due to limited biomass.

Genotoxic and developmental effects in sea urchins are sensitive indicators of effects of genotoxic chemicals.
Anderson S. L., Hose J. E. and Knezovich J. P.
Environ Toxicol Chem. 1994;13(7):1033-41.

Purple sea urchin (Strongylocentrotus purpuratus) gametes and embryos were exposed to three known mutagenic chemicals (phenol, benzidine, and pentachlorophenol) over concentration ranges bracketing the effect levels for fertilization success. Normal development and cytogenic effects (anaphase aberrations) were assessed after the cultures were allowed to develop for 48 h. Using radiolabelled chemicals, we also characterized concentrations in the test water as well as doses in the embryos following 2- and 48-h exposures. We observed dose responses for all chemicals and all responses, except for phenol, which showed no significant effect on development. Fertilization success was never the most sensitive end point. Anaphase aberrations were the most sensitive response for phenol, with an LOEC of 2.5 mg/L exposure concentration. Anaphase aberrations and development were equivalent in sensitivity for benzidine within the tested dose range, and an LOEC of less than 0.1 mg/L was observed. Development was the most sensitive response for pentachlorophenol (LOEC 1 mg/L). The LOEC values for this study were generally lower than comparable data for aquatic life or human health protection. We conclude that genotoxicity and development evaluations should be included in environmental management applications and that tests developed primarily for human health protection do not reliably predict the effects of toxic substances on aquatic life.

Comparison of sublethal and lethal criteria for nine different chemicals in standardized toxicity tests using the earthworm Eisenia andrei.
Van Gestel C. A., Dirven-Van Breemen E. M., Baerselman R., Emans H. J., Janssen J. A., Postuma R. and Van Vliet P. J.
Ecotoxicol Environ Saf. 1992;23(2):206-20.

In this study, the effects of nine different chemicals on the survival, growth, and reproduction of the earthworm species Eisenia andrei were determined using a recently developed method. Earthworms were exposed for 3 weeks to the test chemicals in an artificial soil substrate. Additional data on the acute toxicity of these chemicals were derived from the literature. For some chemicals, cocoon production was the most sensitive parameter (cadmium, chromium, paraquat, fentin, benomyl, phenmedipham), while for others cocoon hatchability was most sensitive (pentachlorophenol, parathion, carbendazim). In the case of parathion, growth of the worms seemed to be even more sensitive than reproduction. As an overall parameter for the effect on earthworm reproduction, the total number of juveniles produced per worm appeared to be a useful parameter. Differences between (acute) LC50 values and the lowest NOEC value for effects on growth and reproduction were different for each chemical. Difference was greatest for cadmium (a factor of greater than 100) and smallest for fentin, benomyl, and pentachlorophenol (a factor of 5-6).

Chronic toxicity of a pure and technical grade pentachlorophenol to Daphnia magna.
Stephenson G. L., Kaushik N. K. and Solomon K. R.
Arch Environ Contam Toxicol. 1991;21(3):388-94.

Chronic toxicity test procedures (static, with renewal) were used to determine the chronic toxicity of sublethal concentrations of a technical formulation of pentachlorophenol (PCP) and pure pentachlorophenol to Daphnia magna. Test organisms 48 +/- 12 h old were exposed for their entire lifespan (i.e., until death) to 0.01, 0.05, 0.1 and 0.5 mg technical PCP/L and 0.01, 0.087 and 0.1 mg pure PCP/L. Criteria used to assess chronic toxicity were mean time to appearance of the primiparous instar in the brood chamber, mean number of days to release of the first brood, mean number of broods produced per female, mean brood size per female, mean number of reproductive days, mean number of young produced per reproductive day per female and survivorship. Pentachlorophenol differentially affected maturation and reproduction but not survivorship or longevity. Mean number of broods produced per daphnid, length of the reproductive period, longevity and survivorship were insensitive criteria relative to mean time to appearance of the primiparous instar, time to

release of first brood, brood size, and number of young produced per daphnid per reproductive day. Generally, there was little difference in toxicity of the three concentrations of pure PCP, for they significantly reduced mean brood size and rate of reproduction of young and significantly but differentially affected maturation. Technical PCP, at the highest concentration of 0.5 mg/L, significantly reduced mean brood size and the rate of production of young, and significantly delayed both time to appearance of the primiparous instar and release of the first brood. When differences in toxicity occurred, generally, pure PCP was more toxic than comparable concentrations of technical PCP. Although enhanced maturation was observed there was no compensatory reproduction.(ABSTRACT TRUNCATED AT 250 WORDS)

Effects of selected chemicals on the glutathione status in the male reproductive system of rats.
Gandy J., Millner G. C., Bates H. K., Casciano D. A. and Harbison R. D.
J Toxicol Environ Health. 1990;29(1):45-57.

Previous studies have suggested a significant role for reproductive tract glutathione in protecting against chemical-induced germ-cell mutations. Therefore, a number of compounds were tested for their ability to perturb glutathione levels in the testes and epididymides as well as liver following single acute dosages to rats. Phorone (250 mg/kg), isophorone (500 mg/kg), and diethyl maleate (500 mg/kg) significantly reduced glutathione in the liver and in both reproductive organs examined. Methyl iodide (100 mg/kg), trimethyl phosphate (600 mg/kg), naphthalene (500 mg/kg), acetaminophen (1500 mg/kg), and pentachlorophenol (25 mg/kg) affected hepatic and epididymal glutathione, but had little or no effect on testicular levels. The ability of isophorone to enhance the covalent binding of tritiated ethyl methanesulfonate (3H-EMS) to spermatocytes was assessed. Perturbation of reproductive tract glutathione by isophorone treatment significantly enhanced the extent of 3H-EMS-induced binding to sperm heads. The temporal pattern of ethylations in sperm heads was consistent with the stage of sperm development known to be susceptible to ethylations by EMS. Therefore, chemical-induced lowering of glutathione in the male reproductive tract may be a mechanism for potentiation of chemical-induced germ-cell mutations.

Development of a standardized reproduction toxicity test with the earthworm species Eisenia fetida andrei using copper, pentachlorophenol and 2,4-dichloroaniline.
van Gestel C. A., van Dis W. A., van Breemen E. M. and Sparenburg P. M.
Ecotoxicol Environ Saf. 1989;18(3):305-12.

This article describes a standardized test method for determining the effect of chemical substances on the reproduction of the earthworm Eisenia fetida andrei. It is based on the existing guidelines for acute toxicity testing with earthworms, and for reasons of standardization the same artificial soil substrate and earthworm species were chosen as prescribed by these guidelines. After being preconditioned for one week in untreated soil, earthworms are exposed to the chemical substances for 3 weeks. The number of cocoons produced is determined, and cocoons are incubated in untreated artificial soil for 5 weeks to assess hatchability. Results are presented from toxicity experiments with pentachlorophenol, copper, and 2,4-dichloroaniline. For these compounds no-effect levels (NEL) for cocoon production were 32, 60-120, and 56 mg.kg-1 dry soil, respectively. Hatching of cocoons was influenced by pentachlorophenol (NEL, 10 mg.kg-1), but not by copper and dichloroaniline. Following exposure, earthworms were incubated in clean soil again to study the possibility of recovery of cocoon production. For copper and dichloroaniline earthworms did recover cocoon production to a level as high as the control level or even higher; in case of pentachlorophenol, cocoon production was still reduced after 3 weeks in clean soil.

Effects of phenol, 2,4-dimethylphenol, 2,4-dichlorophenol, and pentachlorophenol on embryo, larval, and early-juvenile fathead minnows (Pimephales promelas).
Holcombe G. W., Phipps G. L. and Fiandt J. T.
Arch Environ Contam Toxicol. 1982;11(1):73-8.

Embryos of fathead minnows were more resistant to phenol, 2,4-dimethylphenol (2,4-DMP), 2,4-dichlorophenol (2,4-DCP), and pentachlorophenol (PCP) than were larval or juvenile life stages. Growth of 28-day-old fish was the most sensitive indicator of stress during exposures to phenol, 2,4-DMP, and PCP, whereas survival was the most sensitive indicator of toxic effects from 2,4-DCP exposure. Based on these effects, the estimated maximum acceptable toxicant concentration for fathead minnows in Lake Superior water lies between 1,830 and 3,570 micrograms/L for phenol; 1,970 and 3,110 micrograms/L for 2,4-DMP; 290 and 460 micrograms/L for 2,4-DCP; and 44.9 and 73.0 micrograms/L for PCP.

E. Titles only (abstracts not available)

Effect of sublethal pentachlorophenol on early oogenesis in maturing female rainbow trout (Salmo gairdneri).
Nagler J. J., Aysola P. and Ruby S. M.
Arch Environ Contam Toxicol. 1986;15(5):549-55.

TERATOGENIC POTENTIAL OF PURIFIED PCP AND PCA IN SUBCHRONICALLY EXPOSED SPRAGUE-DAWLEY RATS.
Welsh J. J., Collins T. X., Black T. N., Graham S. L. and Odonnell M. W. J. R.
J Am Coll Toxicol. 1985;4(6):372-73.

EFFECT OF PH ON PENTACHLOROPHENOL TOXICITY TO EMBRYOS AND LARVAE OF ZEBRAFISH (BRACHYDANIO RERIO).
Dave G.
Bull Environ Contam Toxicol. 1984;33:621-30.

EFFECT OF PENTACHLOROPHENOL ON THE GROWTH AND MORTALITY OF EMBRYONIC AND JUVENILE STEELHEAD TROUT.
Dominguez S. E. and Chapman G. A.
Arch Environ Contam Toxicol. 1984;13:739-43.

TEMPERATURE-INDUCED CHANGES IN PENTACHLOROPHENOL CHRONIC TOXICITY TO EARLY LIFE STAGES OF RAINBOW TROUT.
Hodson P. V. and Blunt B. R.
Aquat Toxicol. 1981;1:113-27.

EFFECTS OF PENTACHLOROPHENOL ON FETAL AND POSTPARTUM DEVELOPMENT IN THE RAT.
Chou K. and Cook R. M.
Fed Proc Fed Am Soc Exp Biol. 1979;38(868).

RESULTS OF TWO-YEAR TOXICITY AND REPRODUCTION STUDIES ON PENTACHLOROPHENOL IN RATS.
Schwetz B. A., Quast J. F., Keeler P. A., Humiston C. G. and Kociba R. J.
Environ Sci Res. 1978;12:301-09.

CHRONIC TOXICITY AND REPRODUCTION STUDIES IN RATS GIVEN PENTACHLOROPHENOL BY THE DIETARY ROUTE.
Schwetz B. A., Quast J. F., Keeler P. A., Humiston C. G. and Kociba R. J.
Toxicol Appl Pharmacol. 1977;41(138).

The effect of purified and commercial grade pentachlorophenol on rat embryonal and fetal development.
Schwetz B. A., Keeler P. A. and Gehring P. J.
Toxicol Appl Pharmacol. 1974;28(1):151-61.

FETOTOXIC EFFECTS OF PENTACHLOROPHENOL IN THE GOLDEN SYRIAN HAMSTER.
Hinkle D. K.
Toxicol Appl Pharmacol. 1973;25(455).

EFFECT OF TETRACHLOROPHENOL AND PENTACHLOROPHENOL ON RAT EMBRYONAL AND FETAL DEVELOPMENT.
Schwetz B. A. and Gehring P. J.
Toxicol Appl Pharmacol. 1973;25(455).

APPENDIX: TETRACHLOROETHYLENE

Tetrachloroethylene, also known as perchloroethylene (perc: CAS# 127-18-4), is a chlorinated hydrocarbon compound used in dry cleaning and textile processing, metal degreasing operations, and as a chemical intermediate.

This document presents a compilation of abstracts of articles on the developmental and reproductive toxicity of tetrachloroethylene identified during our epidemiological screen and subsequent toxicological evaluation. OEHHA originally screened tetrachloroethylene in 2007 but there was not sufficient human data available for tetrachloroethylene to pass the screen at that time. We applied an epidemiologic data screen on tetrachloroethylene in 2015. The criterion for passing this screen is the existence of two or more analytical epidemiologic studies judged to be of adequate quality that reported increased risk of adverse developmental or reproductive outcomes. Tetrachloroethylene passed the epidemiologic screen. We also conducted a preliminary toxicological evaluation searching for relevant studies, including animal studies.

OEHHA used the information in this document to select tetrachloroethylene for presentation to the Developmental and Reproductive Toxicant Identification Committee as a possible candidate for Committee consideration. The abstracts compiled below are from epidemiologic and animal toxicity studies reporting on developmental and reproductive sequelae related to exposure to tetrachloroethylene, as well as other relevant investigations (e.g., *in vitro* studies or studies in non-mammalian animal species).

Based on a review of abstracts of the following studies, the chemical passed the epidemiologic screen.

- Thirteen epidemiologic studies of tetrachloroethylene reporting statistically significant increased risk of adverse developmental or reproductive outcomes were identified, five of which were analytical studies of adequate quality. Ten additional epidemiologic studies reported increased risk of adverse developmental or reproductive outcomes; however, the findings were not statistically significant. Five epidemiologic studies reporting no increased risk of adverse developmental or reproductive outcomes were identified. Five related articles were also identified.

In addition, the following animal toxicity studies were identified.

- Four animal studies of tetrachloroethylene reporting reproductive or developmental toxicity were identified. No animal studies or meeting abstracts reporting no reproductive or developmental toxicity were identified. One study with unclear findings, ten related articles, and four studies with no abstracts were also identified.

Contents

I. Epidemiologic DART Studies

A. Studies reporting increased risk of adverse developmental or reproductive outcomes

i. Studies which were statistically significant

In utero exposure to toxic air pollutants and risk of childhood autism.
von Ehrenstein O. S., Aralis H., Cockburn M. and Ritz B.
Epidemiology (Cambridge, Mass). 2014;25(6):851-8.

BACKGROUND: Genetic and environmental factors are believed to contribute to the development of autism, but relatively few studies have considered potential environmental risks. Here, we examine risks for autism in children related to in utero exposure to monitored ambient air toxics from urban emissions.
METHODS: Among the cohort of children born in Los Angeles County, California, 1995-2006, those whose mothers resided during pregnancy in a 5-km buffer around air toxics monitoring stations were included (n = 148,722). To identify autism cases in this cohort, birth records were linked to records of children diagnosed with primary autistic disorder at the California Department of Developmental Services between 1998 and 2009 (n = 768). We calculated monthly average exposures during pregnancy for 24 air toxics selected based on suspected or known neurotoxicity or neurodevelopmental toxicity. Factor analysis helped us identify the correlational structure among air toxics, and we estimated odds ratios (ORs) for autism from logistic regression analyses.
RESULTS: Autism risks were increased per interquartile range increase in average concentrations during pregnancy of several correlated toxics mostly loading on 1 factor, including 1,3-butadiene (OR = 1.59 [95% confidence interval = 1.18-2.15]), meta/para-xylene (1.51 [1.26-1.82]), other aromatic solvents, lead (1.49 [1.23-1.81]), perchloroethylene (1.40 [1.09-1.80]), and formaldehyde (1.34 [1.17-1.52]), adjusting for maternal age, race/ethnicity, nativity, education, insurance type, parity, child sex, and birth year.
CONCLUSIONS: Risks for autism in children may increase following in utero exposure to ambient air toxics from urban traffic and industry emissions, as measured by community-based air-monitoring stations.

[*]Prenatal drinking-water exposure to tetrachloroethylene and ischemic placental disease: a retrospective cohort study.
Carwile J. L., Mahalingaiah S., Winter M. R. and Aschengrau A.
Environ Health. 2014;13:72.

BACKGROUND: Prenatal drinking water exposure to tetrachloroethylene (PCE) has been previously related to intrauterine growth restriction and stillbirth. Pathophysiologic and epidemiologic evidence linking these outcomes to certain other pregnancy complications, including placental abruption, preeclampsia, and small-for-gestational-age (SGA) (i.e., ischemic placental diseases), suggests that PCE exposure may also be associated with these events. We examined whether prenatal exposure to PCE-contaminated drinking water was associated with overall or individual ischemic placental diseases. METHODS: Using a retrospective cohort design, we compared 1,091 PCE-exposed and 1,019 unexposed pregnancies from 1,766 Cape Cod, Massachusetts women. Exposure between 1969 and 1990 was estimated using water distribution system modeling software. Data on birth weight and gestational age were obtained from birth certificates; mothers self-reported pregnancy complications. RESULTS: Of 2,110 eligible pregnancies, 9% (N = 196) were complicated by >/=1 ischemic placental disease. PCE exposure was not associated with overall ischemic placental disease (for PCE >/= sample median vs. no exposure, risk ratio (RR): 0.90; 95% confidence interval (CI): 0.65, 1.24), preeclampsia (RR: 0.36; 95% CI: 0.12-1.07), or SGA (RR: 0.98; 95% CI: 0.66-1.45). However, pregnancies with PCE exposure >/= the sample median had 2.38-times the risk of stillbirth >/=27 weeks gestation (95% CI: 1.01, 5.59), and 1.35-times of the risk of placental abruption (95% CI: 0.68, 2.67) relative to unexposed pregnancies. CONCLUSIONS: Prenatal PCE exposure was not associated with overall ischemic placental disease, but may increase risk of stillbirth.

[*]Prenatal and early childhood exposure to tetrachloroethylene and adult vision.
Getz K. D., Janulewicz P. A., Rowe S., Weinberg J. M., Winter M. R., Martin B. R., Vieira V. M., White R. F. and Aschengrau A.
Environ Health Perspect. 2012;120(9):1327-32.

BACKGROUND: Tetrachloroethylene (PCE; or perchloroethylene) has been implicated in visual impairments among adults with occupational and environmental exposures as well as children born to women with occupational exposure during pregnancy. OBJECTIVES: Using a population-based retrospective cohort study, we examined the

[*] denotes that, from review of the abstract, the study is considered to have met the criteria for evidence of an adverse developmental or reproductive effect associated with exposure to the chemical.

association between prenatal and early childhood exposure to PCE-contaminated drinking water on Cape Cod, Massachusetts, and deficits in adult color vision and contrast sensitivity. METHODS: We estimated the amount of PCE that was delivered to the family residence from participants' gestation through 5 years of age. We administered to this now adult study population vision tests to assess acuity, contrast sensitivity, and color discrimination. RESULTS: Participants exposed to higher PCE levels exhibited lower contrast sensitivity at intermediate and high spatial frequencies compared with unexposed participants, although the differences were generally not statistically significant. Exposed participants also exhibited poorer color discrimination than unexposed participants. The difference in mean color confusion indices (CCI) was statistically significant for the Farnsworth test but not Lanthony's D-15d test [Farnsworth CCI mean difference = 0.05, 95% confidence interval (CI): 0.003, 0.10; Lanthony CCI mean difference = 0.07, 95% CI: -0.02, 0.15]. CONCLUSIONS: Prenatal and early childhood exposure to PCE-contaminated drinking water may be associated with long-term subclinical visual dysfunction in adulthood, particularly with respect to color discrimination. Further investigation of this association in similarly exposed populations is necessary.

***Occurrence of mental illness following prenatal and early childhood exposure to tetrachloroethylene (PCE)-contaminated drinking water: a retrospective cohort study**.
Aschengrau A., Weinberg J. M., Janulewicz P. A., Romano M. E., Gallagher L. G., Winter M. R., Martin B. R., Vieira V. M., Webster T. F., White R. F. and Ozonoff D. M. Environ Health. 2012;11:2.

BACKGROUND: While many studies of adults with solvent exposure have shown increased risks of anxiety and depressive disorders, there is little information on the impact of prenatal and early childhood exposure on the subsequent risk of mental illness. This retrospective cohort study examined whether early life exposure to tetrachloroethylene (PCE)-contaminated drinking water influenced the occurrence of depression, bipolar disorder, post-traumatic stress disorder, and schizophrenia among adults from Cape Cod, Massachusetts. METHODS: A total of 1,512 subjects born between 1969 and 1983 were studied, including 831 subjects with both prenatal and early childhood PCE exposure and 547 unexposed subjects. Participants completed questionnaires to gather information on mental illnesses, demographic and medical characteristics, other sources of solvent exposure, and residences from birth through

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1990. PCE exposure originating from the vinyl-liner of water distribution pipes was assessed using water distribution system modeling software that incorporated a leaching and transport algorithm. RESULTS: No meaningful increases in risk ratios (RR) for depression were observed among subjects with prenatal and early childhood exposure (RR: 1.1, 95% CI: 0.9-1.4). However, subjects with prenatal and early childhood exposure had a 1.8-fold increased risk of bipolar disorder (N = 36 exposed cases, 95% CI: 0.9-1.4), a 1.5-fold increased risk post-traumatic stress disorder (N = 47 exposed cases, 95% CI: 0.9-2.5), and a 2.1-fold increased risk of schizophrenia (N = 3 exposed cases, 95% CI: 0.2-20.0). Further increases in the risk ratio were observed for bipolar disorder (N = 18 exposed cases, RR; 2.7, 95% CI: 1.3-5.6) and post-traumatic stress disorder (N = 18 exposed cases, RR: 1.7, 95% CI: 0.9-3.2) among subjects with the highest exposure levels. CONCLUSIONS: The results of this study provide evidence against an impact of early life exposure to PCE on the risk of depression. In contrast, the results provide support for an impact of early life exposure on the risk of bipolar disorder and post-traumatic stress disorder. The number of schizophrenia cases was too small to draw reliable conclusions. These findings should be confirmed in investigations of other similarly exposed populations.

[*]Affinity for risky behaviors following prenatal and early childhood exposure to tetrachloroethylene (PCE)-contaminated drinking water: a retrospective cohort study.
Aschengrau A., Weinberg J. M., Janulewicz P. A., Romano M. E., Gallagher L. G., Winter M. R., Martin B. R., Vieira V. M., Webster T. F., White R. F. and Ozonoff D. M. Environ Health. 2011;10:102.

BACKGROUND: Many studies of adults with acute and chronic solvent exposure have shown adverse effects on cognition, behavior and mood. No prior study has investigated the long-term impact of prenatal and early childhood exposure to the solvent tetrachloroethylene (PCE) on the affinity for risky behaviors, defined as smoking, drinking or drug use as a teen or adult. OBJECTIVES: This retrospective cohort study examined whether early life exposure to PCE-contaminated drinking water influenced the occurrence of cigarette smoking, alcohol consumption, and drug use among adults from Cape Cod, Massachusetts. METHODS: Eight hundred and thirty-one subjects with prenatal and early childhood PCE exposure and 547 unexposed subjects were studied. Participants completed questionnaires to gather information on risky behaviors as a teenager and young adult, demographic characteristics, other

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sources of solvent exposure, and residences from birth through 1990. PCE exposure was estimated using the U.S. EPA's water distribution system modeling software (EPANET) that was modified to incorporate a leaching and transport model to estimate PCE exposures from pipe linings. RESULTS: Individuals who were highly exposed to PCE-contaminated drinking water during gestation and early childhood experienced 50-60% increases in the risk of using two or more major illicit drugs as a teenager or as an adult (Relative Risk (RR) for teen use = 1.6, 95% CI: 1.2-2.2; and RR for adult use = 1.5, 95% CI: 1.2-1.9). Specific drugs for which increased risks were observed included crack/cocaine, psychedelics/hallucinogens, club/designer drugs, Ritalin without a prescription, and heroin (RRs:1.4-2.1). Thirty to 60% increases in the risk of certain smoking and drinking behaviors were also seen among highly exposed subjects. CONCLUSIONS: The results of this study suggest that risky behaviors, particularly drug use, are more frequent among adults with high PCE exposure levels during gestation and early childhood. These findings should be confirmed in follow-up investigations of other exposed populations.

***Tetrachloroethylene exposure and risk of schizophrenia: offspring of dry cleaners in a population birth cohort, preliminary findings**.
Perrin M. C., Opler M. G., Harlap S., Harkavy-Friedman J., Kleinhaus K., Nahon D., Fennig S., Susser E. S. and Malaspina D.
Schizophr Res. 2007;90(1-3):251-4.

Tetrachloroethylene is a solvent used in dry cleaning with reported neurotoxic effects. Using proportional hazard methods, we examined the relationship between parental occupation as a dry cleaner and risk for schizophrenia in a prospective population-based cohort of 88,829 offspring born in Jerusalem from 1964 through 1976, followed from birth to age 21-33 years. Of 144 offspring whose parents were dry cleaners, 4 developed schizophrenia. We observed an increased incidence of schizophrenia in offspring of parents who were dry cleaners (RR=3.4, 95% CI, 1.3-9.2, p=0.01). Tetrachloroethylene exposure warrants further investigation as a risk factor for schizophrenia.

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The influence of maternal exposure to volatile organic compounds on the cytokine secretion profile of neonatal T cells.

Lehmann I., Thoelke A., Rehwagen M., Rolle-Kampczyk U., Schlink U., Schulz R., Borte M., Diez U. and Herbarth O.
Environ Toxicol. 2002;17(3):203-10.

Indoor VOC (volatile organic compound) exposure has been shown to be correlated with airway symptoms and allergic manifestations in children. An investigation was conducted within an ongoing birth cohort study (LISA: Lifestyle-Immune System-Allergy) of the association between maternal exposure to VOCs and immune status at birth, in particular the cytokine secretion profile of cord-blood T cells. In a randomly selected group of 85 neonates, cytokine-producing cord-blood T cells were analyzed using intracellular cytokine detection. VOC exposure was measured in children's dwellings by passive sampling, while parents were asked to complete questionnaires about possible sources of VOC exposure. Adjusted odds ratios (ORs) were calculated by logistic regression based on categorized quartiles. A positive association was found between elevated percentages of interleukin-4-producing (IL-4) type 2 T cells and exposure to naphthalene (OR = 2.9) and methylcyclopentane (OR = 3.3). Exposure to tetrachloroethylene was associated with reduced percentages of interferon-gamma-producing (IFN-gamma) type 1 T cells (OR = 2.9). In addition, smoking during pregnancy was correlated with a higher indoor air concentration of naphthalene (OR = 3.8), new carpets in infants' bedrooms with elevated methylcyclopentane concentrations (OR = 4.1), and home renovation with a higher trichloroethylene burden (OR = 4.9). Our data suggest that maternal exposure to VOC may have an influence on the immune status of the newborn child.

Tetrachloroethylene in drinking water and birth outcomes at the US Marine Corps Base at Camp Lejeune, North Carolina.
Sonnenfeld N., Hertz-Picciotto I. and Kaye W. E.
Am J Epidemiol. 2001;154(10):902-8.

A study of mean birth weight, small-for-gestational-age infants, and preterm birth was conducted at the US Marine Corps Base at Camp Lejeune, North Carolina, where drinking water was contaminated with volatile organic compounds. Tetrachloroethylene (PCE) was the predominant contaminant. The authors used multiple linear and logistic regression to analyze 1968-1985 data from 11,798 birth certificates. Overall, at most weak associations were observed between PCE exposure and study outcomes. However, associations were found between PCE exposure and birth-weight outcomes

for infants of older mothers and mothers with histories of fetal loss. Adjusted mean birth-weight differences between PCE-exposed and unexposed infants were -130 g (90% confidence interval (CI): -236, -23) for mothers aged 35 years or older and -104 g (90% CI: -174, -34) for mothers with two or more previous fetal losses. Adjusted odds ratios for PCE exposure and small-for-gestational-age infants were 2.1 (90% CI: 0.9, 4.9) for older mothers and 2.5 (90% CI: 1.5, 4.3) for mothers with two or more prior fetal losses. These results suggest that some fetuses may be more vulnerable than others to chemical insult.

Spontaneous abortion in dry cleaning workers potentially exposed to perchloroethylene.
Doyle P., Roman E., Beral V. and Brookes M.
Occup Environ Med. 1997;54(12):848-53.

OBJECTIVES: To investigate the association between spontaneous abortion and work within dry cleaning units in the United Kingdom where the solvent perchloroethylene is used. METHODS: A retrospective occupational study of reproductive outcome in 7305 women aged 16 and 45 years, who were currently or previously employed in dry cleaning or laundry units in the United Kingdom. Data on workplace exposures and reproductive outcome were obtained by postal survey. A sample of reported spontaneous abortions was validated from medical records. Machine operator versus non-operator was used as a surrogate for exposure to perchloroethylene in dry cleaning units as no data on individual doses were available for women in this study. RESULTS: The response rate was higher for current workers of dry cleaning units (78%), than for past workers of dry cleaning units (46%). Similarly, the response for current laundry workers (65%) was higher than that for past laundry workers (40%). Overall, the reproductive characteristics of the respondents were similar to expectation. Examination of exposure at the time of pregnancy, however, showed that the rate of spontaneous abortion varied according to the type of work the women did during the pregnancy or in the three months before conception: being lowest for pregnancies not exposed to either dry cleaning or laundry work (10.9%), higher for those exposed to laundry work (13.4%), and higher still for those exposed to dry cleaning work (14.8%). Within the group of pregnancies exposed to dry cleaning, the proportion was higher if the woman reported that she worked as an operator at the time of the pregnancy (17.1%) rather than as a non-operator (11.6%). Adjusted odds ratios for the period 1980-95 showed that the risk was over 50% higher in operators than non-operators (p = 0.04). The physical demands of the two jobs are likely to be similar. A higher risk was found when work as a dry cleaning operator was compared with no work in either dry cleaning or laundry units during pregnancy. Exposure to dry cleaning as a non-operator was not associated with

any excess risk. CONCLUSIONS: Women who worked in dry cleaning shops at the time of their pregnancy or in the three months before who described themselves as operators were about half as likely again to report that their pregnancy ended in a spontaneous abortion than women who described themselves as non-operators.

Public drinking water contamination and birth outcomes.
Bove F. J., Fulcomer M. C., Klotz J. B., Esmart J., Dufficy E. M. and Savrin J. E.
Am J Epidemiol. 1995;141(9):850-62.

The effects of public drinking water contamination on birth outcomes were evaluated in an area of northern New Jersey. After excluding plural births and chromosomal defects, 80,938 live births and 594 fetal deaths that occurred during the period 1985-1988 were studied. Information on birth outcome status and maternal risk factors was obtained from vital records and the New Jersey Birth Defects Registry. Monthly exposures during pregnancy were estimated for all births using tap water sample data. Odds ratios of > or = 1.50 were found for the following: total trihalomethanes with small for gestational age, central nervous system defects, oral cleft defects, and major cardiac defects; carbon tetrachloride with term low birth weight, small for gestational age, very low birth weight, total surveillance birth defects, central nervous system defects, neural tube defects, and oral cleft defects; trichloroethylene with central nervous system defects, neural tube defects, and oral cleft defects; tetrachloroethylene with oral cleft defects; total dichloroethylenes with central nervous system defects and oral cleft defects; benzene with neural tube defects and major cardiac defects; and 1,2-dichloroethane with major cardiac defects. Total trihalomethane levels > 100 ppb reduced birth weight among term births by 70.4 g. By itself, this study cannot resolve whether the drinking water contaminants caused the adverse birth outcomes; therefore, these findings should be followed up utilizing available drinking water contamination databases.

Exposure to organic solvents and adverse pregnancy outcome.
Windham G. C., Shusterman D., Swan S. H., Fenster L. and Eskenazi B.
American journal of industrial medicine. 1991;20(2):241-59.

In a large case-control study (n = 1,926) of spontaneous abortion (SAB), exposure to solvents was ascertained by a telephone interview that asked about occupational use of 18 specific solvents or products, as well as an open-ended "other" solvent category. The adjusted odds ratio for use of any solvent was 1.1 (0.8, 1.5). Solvents for which at least a doubled crude risk of SAB was found included perchlorethylene (OR = 4.7, 95% CI = 1.1, 21.1), trichloroethylene (OR = 3.1, CI = 0.9, 10.4), and paint thinners (OR = 2.3, CI = 1.0, 5.1). Comparing exposure greater than 10 hours per week versus less did

not show consistent dose-response effects. By solvent class, an association was seen with aliphatic solvents (adjusted OR = 1.8, 95% CI = 1.1, 3.0), but there was no dose-response effect by hours of use. Household use of solvent-containing products was generally not strongly associated with SAB, nor did it appear to confound the association seen with occupational use. From this and other studies, occupational exposure to at least some solvents appears associated with SAB. The associations of solvent exposure and fetal growth among liveborn offspring of controls was also examined.

A study of the effect of perchloroethylene exposure on semen quality in dry cleaning workers.
Eskenazi B., Wyrobek A. J., Fenster L., Katz D. F., Sadler M., Lee J., Hudes M. and Rempel D. M.
Am J Ind Med. 1991;20(5):575-91.

The purpose of this investigation was to determine the effects of perchloroethylene (PCE) exposure on human semen quality. We compared the semen quality of 34 dry cleaners with that of 48 laundry workers. We examined the relationships of 17 semen parameters to expired air levels of PCE and to an index of exposure based on job tasks in the last three months. The average sperm concentration was over 80 million for both dry cleaners and laundry workers, but approximately one-quarter of each group was oligospermic. The overall percentage of abnormal forms was similar for the two groups; however, sperm of dry cleaners were significantly more likely to be round ($t = -3.29$, $p = 0.002$) and less likely to be narrow ($t = 2.35$, $p = 0.02$) than the sperm of laundry workers. These effects were dose-related to expired air levels and to the exposure index after controlling for potential confounders (e.g., heat exposure). The average percent motile sperm for both groups was slightly over 60%; however, sperm of dry cleaners tended to swim with greater amplitude of lateral head displacement (ALH) than those of laundry workers ($t = -1.73$, $p = 0.09$), and level of PCE in expired air was a significant predictor of ALH in the multiple regression model ($t = 2.00$, $p = 0.05$). In addition, exposure index was a significant negative predictor of the sperm linearity parameter ($t = -2.57$, $p = 0.01$). These results suggest that occupational exposures to PCE can have subtle effects on sperm quality. Additional analyses are required to determine whether these effects are associated with changes in fertility.

Spontaneous abortions and congenital malformations among women exposed to tetrachloroethylene in dry cleaning.

Kyyronen P., Taskinen H., Lindbohm M. L., Hemminki K. and Heinonen O. P.
J Epidemiol Community Health. 1989;43(4):346-51.

STUDY OBJECTIVE: The aim of the study was to determine whether exposure to tetrachloroethylene during the first trimester of pregnancy has harmful effects on pregnancy outcome. DESIGN: The study used record linkage identification of cases and case-control comparison. SETTING: The study involved dry cleaner and laundry workers throughout Finland who had become pregnant during the study period. Controls were age matched but otherwise unselected women giving birth to normal babies in the study period. SUBJECTS: Cases were defined as women who had been treated for spontaneous abortion or had delivered a malformed child. Out of 5700 workers nearly half had been pregnant during the study period. One pregnancy only was randomly selected for study per worker, and the final study population was 247 women with spontaneous abortions and 33 with malformed infants. Three age matched controls were selected for each abortion case and five for each malformation case. MEASUREMENTS AND MAIN RESULTS: Three women out of four had worked in early pregnancy. Exposure information was collected from 1108 women by mailed questionnaires, with a 77% response, and was partly confirmed by biological monitoring data. Exposure to tetrachloroethylene was found to be significantly associated with spontaneous abortions (odds ratio 3.6, p less than 0.05). CONCLUSION: The findings, together with other available data, indicate that exposure of pregnant women to tetrachloroethylene needs to be minimised.

ii. Studies which were not statistically significant

Long-term health effects of early life exposure to tetrachloroethylene (PCE)-contaminated drinking water: a retrospective cohort study.
Aschengrau A., Winter M. R., Vieira V. M., Webster T. F., Janulewicz P. A., Gallagher L. G., Weinberg J. and Ozonoff D. M.
Environ Health. 2015;14:36.

BACKGROUND: While adult exposure to PCE is known to have toxic effects, there is little information on the long-term impact of prenatal and early childhood exposure. We undertook a retrospective cohort study to examine the effects of their early life exposure to PCE-contaminated drinking water. This retrospective cohort study examined whether

prenatal and early childhood exposure to PCE-contaminated drinking water influenced the risk of a variety of chronic conditions among adults who were born between 1969 and 1983 in the Cape Cod area of Massachusetts. METHODS: Eight hundred and thirty-one participants with prenatal and early childhood PCE exposure and 547 unexposed participants were studied. Individuals completed questionnaires to gather information on demographic characteristics, chronic conditions, and other sources of solvent exposure. The location of residences from birth through 1990 were used to estimate PCE exposure with U.S. EPA's water distribution system modeling software (EPANET) modified to incorporate a leaching and transport model. RESULTS: No associations were observed between early life PCE exposure and current occurrence of obesity, diabetes, cardiovascular disease, hypertension, color blindness, near- and far sightedness and dry eyes. In contrast, a 1.8-fold increased risk of cancer (95% CI: 0.8, 4.0) was seen among individuals with any early life exposure. These results were based on 31 participants (23 exposed and 8 unexposed) who reported cancers at a variety of anatomical sites, particularly the cervix. A 1.5-fold increase in the risk of epilepsy (95% CI: 0.6, 3.6, based on 16 exposed and 7 unexposed participants) was also observed among individuals with any early life exposure that was further increased to 1.8 (95% CI: 0.7, 4.6) among those with exposure at or above the sample median. CONCLUSIONS: These results suggest that the risk of epilepsy and certain types of cancer such as cervical cancer may be increased among adults who were exposed to PCE-contaminated drinking water exposure during gestation and early childhood. These findings should be interpreted cautiously because of the study limitations and confirmed in follow-up investigations of similarly exposed populations with medically-confirmed diagnoses. This relatively young study population should also be monitored periodically for subsequent changes in disease risk.

Prenatal exposure to air toxics and risk of Wilms' tumor in 0- to 5-year-old children.
Shrestha A., Ritz B., Wilhelm M., Qiu J., Cockburn M. and Heck J. E.
Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine. 2014;56(6):573-8.

OBJECTIVE: To study prenatal air toxic exposure and Wilms' tumor in children. METHODS: We identified 337 Wilms' tumor cases among children younger than 6 years (1988 to 2008) from the California Cancer Registry, randomly selected 96,514 controls from California birth rolls in 20:1 ratio matched to all cancer cases, then linked birth addresses to air monitors within 15 miles to assess exposures. Multiple logistic regressions were applied to estimate effects.

RESULTS: Children prenatally exposed to formaldehyde, polycyclic aromatic hydrocarbons, perchloroethylene, or acetaldehyde in the third trimester had an increased odds of Wilms' tumor per interquartile increase in concentration (odds ratio [95% confidence interval]: 1.28 [1.12 to 1.45], 1.10 [0.99 to 1.22], 1.09 [1.00 to 1.18], 1.25 [1.07 to 1.45], respectively).
CONCLUSIONS: We found positive associations for four air toxics. This is the first study of this kind. Future studies are needed to confirm our findings.

Evaluation of contaminated drinking water and preterm birth, small for gestational age, and birth weight at Marine Corps Base Camp Lejeune, North Carolina: a cross-sectional study.
Ruckart P. Z., Bove F. J. and Maslia M.
Environ Health. 2014;13:99.

BACKGROUND: Births during 1968-1985 at Camp Lejeune were exposed to drinking water contaminated with trichloroethylene (TCE), tetrachloroethylene (PCE), and benzene. METHODS: We conducted a cross-sectional study to evaluate associations between residential prenatal exposure to contaminated drinking water at Camp Lejeune during 1968-1985 and preterm birth, small for gestational age (SGA), term low birth weight (TLBW), and mean birth weight (MBW) deficit. Birth certificates identified mothers residing at Camp Lejeune at delivery. We analyzed exposure data for the entire pregnancy and individual trimesters. For each period examined, births were categorized as unexposed if mothers did not reside at Camp Lejeune or if their residence on base received uncontaminated drinking water. Ground water contaminant fate/transport and distribution system models provided monthly estimated contaminant levels at residences. For PCE and TCE, the exposed group was divided into four levels: < median value, >/= median value, >/=75th percentile, and >/=90th percentile. For benzene, the exposed group was categorized as <1 part per billion (ppb) versus >/=1 ppb because of sparse data. Magnitude of effect estimates and exposure response relationships were used to assess associations. Confidence intervals (CIs) indicated precision of estimates. RESULTS: For the highest TCE exposure category during the entire pregnancy, odds ratios (ORs) were 1.5 (95% CI: 1.2, 1.9) and 1.3 (95% CI: 0.8, 2.2) for SGA and TLBW, respectively, and reduced MBW beta = -78.3 g (95% CI: -115.0, -41.7). The OR =1.3 (95% CI: 1.0, 1.6) for preterm birth and the highest PCE exposure category during the entire pregnancy. Monotonic exposure-response relationships were observed for benzene exposure during the entire pregnancy and TLBW (highest category OR =1.5, 85% CI: 0.9, 2.3). Although a monotonic association between benzene and adjusted MBW difference was also observed (highest category beta = -36.2 g, 95% CI: -72.3, -0.1), the association disappeared when TCE was also

added to the model. We found no evidence suggesting any other associations between outcomes and exposures. CONCLUSION: Findings suggested associations between in utero exposures to TCE and SGA, TLBW and reduced MBW; benzene and TLBW; and PCE and preterm birth.

Evaluation of exposure to contaminated drinking water and specific birth defects and childhood cancers at Marine Corps Base Camp Lejeune, North Carolina: a case-control study.
Ruckart P. Z., Bove F. J. and Maslia M.
Environ Health. 2013;12:104.

BACKGROUND: Drinking water supplies at Marine Corps Base Camp Lejeune were contaminated with trichloroethylene, tetrachloroethylene, benzene, vinyl chloride and trans-1,2-dichloroethylene during 1968 through 1985. METHODS: We conducted a case control study to determine if children born during 1968-1985 to mothers with residential exposure to contaminated drinking water at Camp Lejeune during pregnancy were more likely to have childhood hematopoietic cancers, neural tube defects (NTDs), or oral clefts. For cancers, exposures during the first year of life were also evaluated. Cases and controls were identified through a survey of parents residing on base during pregnancy and confirmed by medical records. Controls were randomly sampled from surveyed participants who had a live birth without a major birth defect or childhood cancer. Groundwater contaminant fate and transport and distribution system models provided estimates of monthly levels of drinking water contaminants at mothers' residences. Magnitude of odds ratios (ORs) was used to assess associations. Confidence intervals (CIs) were used to indicate precision of ORs. We evaluated parental characteristics and pregnancy history to assess potential confounding. RESULTS: Confounding was negligible so unadjusted results were presented. For NTDs and average 1st trimester exposures, ORs for any benzene exposure and for trichloroethylene above 5 parts per billion were 4.1 (95% CI: 1.4-12.0) and 2.4 (95% CI: 0.6-9.6), respectively. For trichloroethylene, a monotonic exposure response relationship was observed. For childhood cancers and average 1st trimester exposures, ORs for any tetrachloroethylene exposure and any vinyl chloride exposure were 1.6 (95% CI: 0.5-4.8), and 1.6 (95% CI: 0.5-4.7), respectively. The study found no evidence suggesting any other associations between outcomes and exposures. CONCLUSION: Although CIs were wide, ORs suggested associations between drinking water contaminants and NTDs. ORs suggested weaker associations with childhood hematopoietic cancers.

Adult neuropsychological performance following prenatal and early postnatal exposure to tetrachloroethylene (PCE)-contaminated drinking water.
Janulewicz P. A., White R. F., Martin B. M., Winter M. R., Weinberg J. M., Vieira V. and Aschengrau A.
Neurotoxicol Teratol. 2012;34(3):350-9.

This population-based retrospective cohort study examined adult performance on a battery of neuropsychological tests in relation to prenatal and early postnatal exposure to tetrachloroethylene (PCE)-contaminated drinking water on Cape Cod, Massachusetts. Subjects were identified through birth records from 1969 through 1983. Exposure was modeled using pipe network information from town water departments, a PCE leaching and transport algorithm, EPANet water flow modeling software, and a Geographic Information System (GIS). Results of crude and multivariate analyses among 35 exposed and 28 unexposed subjects showed no association between prenatal and early postnatal exposure and decrements on tests that assess abilities in the domains of omnibus intelligence, academic achievement or language. The results were suggestive of an association between prenatal and early postnatal PCE exposure and diminished performance on tests that assessed abilities in the domains of visuospatial functioning, learning and memory, motor, attention and mood. Because the sample size was small, most findings were not statistically significant. Future studies with larger sample sizes should be conducted to further define the neuropsychological consequences of early developmental PCE exposure.

Adverse birth outcomes and maternal exposure to trichloroethylene and tetrachloroethylene through soil vapor intrusion in New York State.
Forand S. P., Lewis-Michl E. L. and Gomez M. I.
Environ Health Perspect. 2012;120(4):616-21.

BACKGROUND: Industrial spills of volatile organic compounds (VOCs) in Endicott, New York (USA), have led to contamination of groundwater, soil, and soil gas. Previous studies have reported an increase in adverse birth outcomes among women exposed to VOCs in drinking water. OBJECTIVE: We investigated the prevalence of adverse birth outcomes among mothers exposed to trichloroethylene (TCE) and tetrachloroethylene [or perchloroethylene (PCE)] in indoor air contaminated through soil vapor intrusion. METHODS: We examined low birth weight (LBW), preterm birth, fetal growth restriction, and birth defects among births to women in Endicott who were exposed to VOCs, compared with births statewide. We used Poisson regression to analyze births and

malformations to estimate the association between maternal exposure to VOCs adjusting for sex, mother's age, race, education, parity, and prenatal care. Two exposure areas were identified based on environmental sampling data: one area was primarily contaminated with TCE, and the other with PCE. RESULTS: In the TCE-contaminated area, adjusted rate ratios (RRs) were significantly elevated for LBW [RR = 1.36; 95% confidence interval (CI): 1.07, 1.73; n = 76], small for gestational age (RR = 1.23; 95% CI: 1.03, 1.48; n = 117), term LBW (RR = 1.68; 95% CI: 1.20, 2.34; n = 37), cardiac defects (RR = 2.15; 95% CI: 1.27, 3.62; n = 15), and conotruncal defects (RR = 4.91; 95% CI: 1.58, 15.24; n = 3). In the PCE-contaminated area, RRs for cardiac defects (five births) were elevated but not significantly. Residual socioeconomic confounding may have contributed to elevations of LBW outcomes. CONCLUSIONS: Maternal residence in both areas was associated with cardiac defects. Residence in the TCE area, but not the PCE area, was associated with LBW and fetal growth restriction.

Prenatal exposure to tetrachloroethylene-contaminated drinking water and the risk of congenital anomalies: a retrospective cohort study.
Aschengrau A., Weinberg J. M., Janulewicz P. A., Gallagher L. G., Winter M. R., Vieira V. M., Webster T. F. and Ozonoff D. M.
Environ Health. 2009;8:44.

BACKGROUND: Prior animal and human studies of prenatal exposure to solvents including tetrachloroethylene (PCE) have shown increases in the risk of certain congenital anomalies among exposed offspring. OBJECTIVES: This retrospective cohort study examined whether PCE contamination of public drinking water supplies in Massachusetts influenced the occurrence of congenital anomalies among children whose mothers were exposed around the time of conception. METHODS: The study included 1,658 children whose mothers were exposed to PCE-contaminated drinking water and a comparable group of 2,999 children of unexposed mothers. Mothers completed a self-administered questionnaire to gather information on all of their prior births, including the presence of anomalies, residential histories and confounding variables. PCE exposure was estimated using EPANET water distribution system modeling software that incorporated a fate and transport model. RESULTS: Children whose mothers had high exposure levels around the time of conception had an increased risk of congenital anomalies. The adjusted odds ratio of all anomalies combined among children with prenatal exposure in the uppermost quartile was 1.5 (95% CI: 0.9, 2.5). No meaningful increases in the risk were seen for lower exposure levels. Increases were also observed in the risk of neural tube defects (OR: 3.5, 95% CI: 0.8, 14.0) and oral clefts (OR 3.2, 95% CI: 0.7, 15.0) among offspring with any prenatal exposure. CONCLUSION: The results of this study suggest that the risk of

certain congenital anomalies is increased among the offspring of women who were exposed to PCE-contaminated drinking water around the time of conception. Because these results are limited by the small number of children with congenital anomalies that were based on maternal reports, a follow-up investigation should be conducted with a larger number of affected children who are identified by independent records.

A study of the effect of perchloroethylene exposure on the reproductive outcomes of wives of dry-cleaning workers.
Eskenazi B., Fenster L., Hudes M., Wyrobek A. J., Katz D. F., Gerson J. and Rempel D. M.
Am J Ind Med. 1991;20(5):593-600.

The purpose of this investigation was to compare the reproductive outcomes of wives of men exposed to perchloroethylene in the dry-cleaning industry compared to those of wives of laundry workers. Seventeen female partners of dry cleaners and 32 partners of laundry workers were interviewed. The number of pregnancies and the standardized fertility ratios were similar between the two groups. Wives of dry cleaners did not have higher rates of spontaneous abortions. However, wives of dry cleaners were more than twice as likely to have a history of attempting to become pregnant for more than 12 months or to have sought care for an infertility problem. Cox proportional hazards models indicated that dry-cleaners' wives had half of the per-cycle pregnancy rate of wives of laundry workers, when controlling for other potential confounders (estimated rate ratio of 0.54, 95% C.I. = 0.23, 1.27).

[Chlorinated solvents and fetal damage. Spontaneous abortions, low birth weight and malformations among women employed in the dry-cleaning industry].
Kolstad H. A., Brandt L. P. and Rasmussen K.
Ugeskr Laeger. 1990;152(35):2481-2.

Workers in the dry-cleaning industry are exposed to vapours from the cleaning fluids, primarily perchlorethylene, which is a chlorinated solvent. Animal experiments, short-term tests for mutagenic effects and epidemiological investigations have raised the suspicion that perchlorethylene may cause reproductive failure. This investigation is an attempt to assess the risk for reproductive failure in 886 women exposed to dry-cleaning solvents while employed in dry-cleaning establishments. Twelve spontaneous abortions occurred, one infant was born with malformations and ten infants had low birth weights. A non-significant risk for spontaneous abortion was found among women with the greatest exposure. This estimate is, however, uncertain and the result must be interpreted with care.

Low birthweight, congenital malformations, and spontaneous abortions among dry-cleaning workers in Scandinavia.
Olsen J., Hemminki K., Ahlborg G., Bjerkedal T., Kyyronen P., Taskinen H., Lindbohm M. L., Heinonen O. P., Brandt L., Kolstad H. and et al.
Scand J Work Environ Health. 1990;16(3):163-8.

With a common study protocol, case-referent studies within cohorts were performed in Denmark, Norway, Sweden, and Finland to study reproductive hazards of women doing dry-cleaning work. Due to national differences not all of the studies could follow exactly the same procedures in data collection, but they were all based on the linkage of cohorts of dry-cleaning and laundry workers to national registers of births and reproductive failures. Summary measures from each study were combined without the data being pooled. The most significant finding was an increased risk of spontaneous abortion among the most exposed women in the Finnish data. This finding was only supported by the results of the other studies to a minor degree, and the combined odds ratio had confidence limits which included unity.

B. Studies reporting no increased risk of adverse developmental or reproductive outcomes

Structural Magnetic Resonance Imaging in an adult cohort following prenatal and early postnatal exposure to tetrachloroethylene (PCE)-contaminated drinking water.
Janulewicz P. A., Killiany R. J., White R. F., Martin B. M., Winter M. R., Weinberg J. M. and Aschengrau A.
Neurotoxicol Teratol. 2013;38:13-20.

This population-based retrospective cohort study examined Structural Magnetic Resonance Imaging (MRI) of the brain in relation to prenatal and early postnatal exposure to tetrachloroethylene (PCE)-contaminated drinking water on Cape Cod, Massachusetts. Subjects were identified through birth records from 1969 through 1983. Exposure was modeled using pipe network information from town water departments, a PCE leaching and transport algorithm, EPANet water flow modeling software, and Geographic Information System (GIS) methodology. Brain imaging was performed on 26 exposed and 16 unexposed subjects. Scans were acquired on a Philips 3T whole body scanner using the ADNI T1-weighted MP-RAGE scan. The scans were processed by FreeSurfer version 4.3.1 software to obtain measurements of specific brain regions.

There were no statistically significant differences between exposed and unexposed subjects on the measures of white matter hypointensities (beta: 127.5mm(3), 95% CI: -259.1, 1514.0), white matter volumes (e.g. total cerebral white matter: beta: 21230.0mm(3), 95% CI: -4512.6, 46971.7) or gray matter volumes (e.g. total cerebral gray matter: beta: 11976.0mm(3), 95% CI: -13657.2, 37609.3). The results of this study suggest that exposure to PCE during gestation and early childhood, at the levels observed in this population, is not associated with alterations in the brain structures studied.

Prenatal exposure to tetrachloroethylene-contaminated drinking water and the risk of adverse birth outcomes.
Aschengrau A., Weinberg J., Rogers S., Gallagher L., Winter M., Vieira V., Webster T. and Ozonoff D.
Environ Health Perspect. 2008;116(6):814-20.

BACKGROUND: Prior studies of prenatal exposure to tetrachloroethylene (PCE) have shown mixed results regarding its effect on birth weight and gestational age. OBJECTIVES: In this retrospective cohort study we examined whether PCE contamination of public drinking-water supplies in Massachusetts influenced the birth weight and gestational duration of children whose mothers were exposed before the child's delivery. METHODS: The study included 1,353 children whose mothers were exposed to PCE-contaminated drinking water and a comparable group of 772 children of unexposed mothers. Birth records were used to identify subjects and provide information on the outcomes. Mothers completed a questionnaire to gather information on residential histories and confounding variables. PCE exposure was estimated using EPANET water distribution system modeling software that incorporated a fate and transport model. RESULTS: We found no meaningful associations between PCE exposure and birth weight or gestational duration. Compared with children whose mothers were unexposed during the year of the last menstrual period (LMP), adjusted mean differences in birth weight were 20.9, 6.2, 30.1, and 15.2 g for children whose mothers' average monthly exposure during the LMP year ranged from the lowest to highest quartile. Similarly, compared with unexposed children, adjusted mean differences in gestational age were -0.2, 0.1, -0.1, and -0.2 weeks for children whose mothers' average monthly exposure ranged from the lowest to highest quartile. Similar results were observed for two other measures of prenatal exposure. CONCLUSIONS: These results suggest that prenatal PCE exposure does not have an adverse effect on these birth outcomes at the exposure levels experienced by this population.

Risk of learning and behavioral disorders following prenatal and early postnatal exposure to tetrachloroethylene (PCE)-contaminated drinking water.
Janulewicz P. A., White R. F., Winter M. R., Weinberg J. M., Gallagher L. E., Vieira V., Webster T. F. and Aschengrau A.
Neurotoxicol Teratol. 2008;30(3):175-85.

This population-based retrospective cohort study examined the association between developmental disorders of learning, attention and behavior and prenatal and early postnatal drinking water exposure to tetrachloroethylene (PCE) on Cape Cod, Massachusetts. Subjects were identified through birth records from 1969 through 1983. Exposure was modeled using information from town water departments, a PCE leaching and transport algorithm, EPANet water flow modeling software, and a Geographic Information System (GIS). Mothers completed a questionnaire on disorders of attention, learning and behavior in their children and on potential confounding variables. The final cohort consisted of 2086 children. Results of crude and multivariate analyses showed no association between prenatal exposure and receiving tutoring for reading or math, being placed on an Individual Education Plan, or repeating a school grade (adjusted Odds Ratios (OR)=1.0-1.2). There was also no consistent pattern of increased risk for receiving a diagnosis of Attention Deficit Disorder (ADD) or Hyperactive Disorder (HD), special class placement for academic or behavioral problems, or lower educational attainment. Modest associations were observed for the latter outcomes only in the low exposure group (e.g., adjusted ORs for ADD were 1.4 and 1.0 for low and high exposure, respectively). (All ORs are based on an unexposed referent group.) Results for postnatal exposure through age five years were similar to those for prenatal exposure. We conclude that prenatal and early postnatal PCE exposure is not associated with disorders of attention, learning and behavior identified on the basis of questionnaire responses and at the exposure levels experienced by this population.

Pregnancy outcome among women working in laundries and dry-cleaning shops using tetrachloroethylene.
Ahlborg G., Jr.
Am J Ind Med. 1990;17(5):567-75.

Case-referent studies were performed within two cohorts of women engaged in laundry or dry-cleaning work. The aim was to elucidate if tetrachloroethylene (perchloroethylene) exposure increased the risk of adverse pregnancy outcome (spontaneous abortion, perinatal death, congenital malformations or birth weight less

than 1,500 g). Pregnancies and outcomes were identified in national registers and exposure data were obtained from the women by postal questionnaires. Response rates were 75-88%. Conditional logistic regression analysis of the total material yielded an adjusted odds ratio for tetrachloroethylene exposure during the first trimester of 1.1 (95% confidence interval 0.6-2.0) when several potential confounding factors were accounted for. The total material included few highly exposed pregnancies and a limited number of cases of specific adverse outcomes. Consequently, the results do not invalidate the recommendation that tetrachloroethylene should be handled with caution by women in childbearing ages.

Spontaneous abortions and congenital malformations among the wives of men occupationally exposed to organic solvents.
Taskinen H., Anttila A., Lindbohm M. L., Sallmen M. and Hemminki K.
Scand J Work Environ Health. 1989;15(5):345-52.

A case-referent study nested in a cohort monitored biologically for exposure to six organic solvents (styrene, toluene, xylene, tetrachloroethylene, trichloroethylene, and 1,1,1-trichloroethane) was conducted to investigate the effects of paternal exposure on pregnancy outcome. The pregnancies were identified from medical registers. The exposures of the men during the spermatogenesis preceding the pregnancies and of the women during the first trimester of the pregnancies were obtained with questionnaires, and the available biological monitoring measurements were used in the exposure assessment. Factors which significantly increased the odds ratio of spontaneous abortion were paternal exposure to organic solvents in general, high/frequent exposure to toluene or miscellaneous organic solvents (including thinners), and maternal heavy lifting. No significant association between paternal or maternal exposure and congenital malformations was found, but because of the few cases no firm conclusions can be drawn.

C. Related articles

Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation.
Gentry P. R., Covington T. R. and Clewell H. J., 3rd
Regulatory toxicology and pharmacology : RTP. 2003;38(1):1-16.

In recent years efforts have increased to develop a framework for assessing differences, both pharmacokinetic and pharmacodynamic, between children and adults

for purposes of assessing risk of adverse effects following chemical exposure. The specific goal of this study was to demonstrate an approach for using PBPK modeling to compare maternal and fetal/neonatal blood and tissue dose metrics during pregnancy and lactation. Six chemical classes were targeted to provide a variety of physicochemical properties (volatility, lipophilicity, water solubility), and surrogate chemicals were selected to represent each class (isopropanol, vinyl chloride, methylene chloride, perchloroethylene, nicotine, and TCDD), based on the availability of pharmacokinetic information. These chemicals were also selected to provide different pharmacokinetic characteristics, including metabolic production of stable or reactive intermediates in the liver and competing pathways for metabolism. Changes in dosimetry during pregnancy predicted by the modeling were mainly attributable to the development of enzymatic pathways in the fetus or to changes in tissue composition in the mother and fetus during pregnancy. In general, blood concentrations were lower in the neonate during the lactation period than in the fetus during gestation. This postnatal decrease varied from only a slight change (for TCDD) to approximately four orders of magnitude (for vinyl chloride). As compared to maternal exposure, fetal/neonatal exposures ranged from approximately twice as great (for TCDD) to several orders of magnitude lower (for isopropanol). The results of this study are in general agreement with the analyses of data on pharmaceutical chemicals, which have suggested that the largest difference in pharmacokinetics observed between children and adults is for the perinatal period. The most important factor appears to be the potential for decreased clearance of toxic chemicals in the perinatal period due to immature metabolic enzyme systems, although this same factor can also reduce the risk from reactive metabolites during the same period.

Volatile organic compounds in drinking water and adverse pregnancy outcomes, United States Marine Corps Base, Camp Lejeune, North Carolina.
Sonnenfeld N. L.
NTIS Technical Report. 1998;156540(158).

In 1995, the Agency for Toxic Substances and Disease Registry (ATSDR) began data collection for a study of environmental exposure to volatile organic compounds (VOCs) in drinking water and a variety of adverse pregnancy outcomes at the U.S. Marine Corps Base at Camp Lejeune, Onslow County, North Carolina. This study was undertaken following documentation that environmental exposure to VOCs in drinking water had occurred in the past. At that time, there was no evidence of an increased rate of adverse pregnancy outcomes at Camp Lejeune. However, because fetuses tend to be more sensitive to toxic chemical exposures and many pregnant women had resided in housing areas supplied with contaminated water, it appeared prudent to research the

topic. This report describes a study of past exposure to VOC-contaminated drinking water and mean birth weight (MBW), small for gestational age (SGA), and preterm birth in residents of base family housing at Camp Lejeune. The results were based on analysis of live births to women residing in base family housing when they delivered during the period January 1, 1968, through December 31, 1985. Birth certificates were studied from 6,117 tetrachloroethylene (PCE)-exposed women, 141 short-term trichloroethylene (TCE)-exposed women, 31 long-term TCE-exposed women, and 5,681 unexposed women. The following potential confounders and effect modifiers were evaluated: sex of infant, maternal and paternal ages, maternal race, maternal and paternal education, military pay grade, maternal parity, adequacy of prenatal care, marital status, and year of birth. The influence of timing and duration of exposure on potential effects was also explored by linking family base housing records to birth certificate data. Preterm delivery was not associated with VOC-exposure in any category. For most live births, including all births to women younger than 35 years of age with no prior fetal deaths, there was no association between PCE-contaminated drinking water and MBW or SGA. For the group as a whole, infants whose mothers resided in PCE-exposed areas weighed an average of 24 grams (g) less at birth than infants whose mothers lived in unexposed housing. This difference was too small to be biologically meaningful. After controlling for potential confounders, the overall odds ratio (OR) for PCE and SGA was 1.2 (90% confidence limits (CL): 1.0, 1.3). These results provide reasonable assurance that PCE-contaminated drinking water did not affect the birth weight of infants of mothers who were younger than 35 years of age and had no medical history of fetal death; this accounted for most base residents exposed to PCE. Associations between PCE and the study outcomes were observed in two potentially susceptible subgroups: infants of mothers 35 years of age or older and infants whose mothers had histories of fetal deaths. For older mothers, the adjusted difference in MBW between PCE-exposed and unexposed births was -205 g (90% CL: -333, -78), and the adjusted OR was 4.0 (90% CL: 1.6, 10.2) for PCE exposure and SGA. In mothers who had previously had one or more fetal deaths, the adjusted OR for PCE and SGA was 1.6 (90% CL: 1.2, 2.1). In mothers who had previously had two or more fetal deaths, the differences in MBW and SGA between PCE-exposed and unexposed mothers were much larger, but the number of births to women in this group was fairly small. Because associations in these subgroups were not anticipated, these results should be considered exploratory. They are, however, biologically plausible and deserving of followup. The TCE-exposed groups were both small in number. The difference in adjusted MBW between the long-term TCE-exposed group and the unexposed comparison group was -139 g (90% CL: -277, -1); the OR was 1.5 (90% CL: 0.5, 3.8) for SGA and long-term TCE exposure. This increase was entirely attributable to differences in male infants within the long-term TCE-exposed group. Among males

alone, the OR for SGA was 3.9 (90% CL: 1.1, 11.9) and the difference in MBW was -312 g (90% CL: -540, -85). The short-term TCE-exposed group had a lower prevalence of SGA infants, and MBW was slightly higher overall in this group compared with the unexposed group. The finding and magnitude of reduced birth weight and increased SGA in males within the long-term TCE-exposed group is potentially important. However, the small sample size considerably weakened the evidence for a causal association. Although it is possible to speculate on mechanisms by which such a sex-based difference might arise, this difference was unexpected and could not be explained by known mechanisms of TCE toxicity. These findings warrant followup in a larger TCE-exposed population. ATSDR had intended to analyze fetal death data, but existing records were too incomplete to be useful. In addition to the main analyses, several substudies were conducted and are presented in appendices A and B. Important conclusions from these substudies are (1) the housing record data were complete and should have provided reasonable information regarding length of exposure during pregnancy; (2) abstracting medical records is feasible and might enrich the data quality for the subgroups of study participants for which associations between VOC-exposure and MBW and SGA were noted; (3) a limited amount of birth defects data were available from the birth certificate. These data were inadequate for a formal evaluation of associations between VOC exposure and birth defects. Alternative approaches are recommended to study VOC exposure and birth defects if the question remains an issue of public health interest.

Lactational transfer of volatile chemicals in breast milk.
Fisher J., Mahle D., Bankston L., Greene R. and Gearhart J.
American Industrial Hygiene Association journal. 1997;58(6):425-31.

Lactational transfer of chemicals to nursing infants is a concern for occupational physicians when women who are breast-feeding return to the workplace. Some work environments, such as paint shops, have atmospheric contamination from volatile organic chemicals (VOCs). Very little is known about the extent of exposure a nursing infant may receive from the mother's occupational exposure. A physiologically based pharmacokinetic model was developed for a lactating woman to estimate the amount of chemical that a nursing infant ingests for a given nursing schedule and maternal occupational exposure. Human blood/air and milk/air partition coefficients (PCs) were determined for 19 VOCs. Milk/blood PC values were above 3 for carbon tetrachloride, methylchloroform, perchloroethylene, and 1,4-dioxane, while the remaining 16 chemicals had milk/blood PC values of less than 3. Other model parameters, such as solid tissue PC values, metabolic rate constants, blood flow rates, and tissue volumes were taken from the literature and incorporated into the lactation model. In a simulated

exposure of a lactating woman to a threshold limit value concentration of an individual chemical, only perchloroethylene, bromochloroethane, and 1,4-dioxane exceeded the U.S. Environmental Protection Agency non-cancer drinking water ingestion rates for children. Very little data exists on the pharmacokinetics of lactational transfer of volatile organics. More data are needed before the significance of the nursing exposure pathway can be adequately ascertained. Physiologically based pharmacokinetic models can play an important role in assessing lactational transfer of chemicals.

Effects on reproduction of tri- and tetrachloroethylene.
Danielsson B. R.
NTIS Technical Report. 1990;211314:3-90.

This report is the result of a project established by the Nordic Chemicals Control Group under the Nordic Council of Ministers. The project plan was accepted by the Control Group in September 1987. The intention of the project is to provide a proposal for scientific criteria for classification of reproductive toxic substances. A harmonized system for classification and hazard labelling of reproductive toxic substances and products was considered to be of great importance by the working group. For the evaluation of the proposed criteria the working group decided to assess the reproductive hazards of different compounds based upon current knowledge. In future endeavours, which will assess the reproductive toxic risk of these compounds to humans, the information presented in these reports will be useful.

Validity of exposure data obtained by questionnaire. Two examples from occupational reproductive studies.
Ahlborg G. A., Jr.
Scand J Work Environ Health. 1990;16(4):284-8.

Exposure data from self-administered questionnaires were compared with independent information on occupational exposures in two studies of reproductive outcome. Agreement in the case-referent study concerning dry-cleaning work and tetrachloroethylene exposure was good. However, exposure reporting was indicated to be more accurate for the cases than the referents. Correction for misclassification slightly changed the odds ratio from 1.02 to 1.27 for nonspecific exposure and from 0.92 to 0.82 for tetrachloroethylene exposure. Missing information on the latter exposure was more crucial, since adding the employer information for such exposure increased the risk estimate to 1.24. In a prospective follow-up study, exposure information was validated in a sample of the study population. Reporting of heavy lifting appeared to be fairly correct, whereas the underreporting of chemical exposures was a problem.

Validation of self-reported exposure data is desirable, and the direction and magnitude of possible misclassification bias should be evaluated in each specific situation.

II. Animal DART Studies

A. Studies reporting developmental or reproductive toxicity

Chlorination byproducts induce gender specific autistic-like behaviors in CD-1 mice.
Guariglia S. R., Jenkins E. C., Jr., Chadman K. K. and Wen G. Y.
Neurotoxicology. 2011;32(5):545-53.

In 2000, the Agency for Toxic Substances and Disease Registry (ATSDR) released a report concerning elevated autism prevalence and the presence water chlorination byproducts in the municipal drinking water supply in Brick Township, New Jersey. The ATSDR concluded that it was unlikely that these chemicals, specifically chloroform, bromoform (Trihalomethanes; THMs) and tetrachloroethylene (Perchloroethylene; PCE) had contributed to the prevalence of autism in this community based upon correlations between timing of exposure and/or concentration of exposure. The ATSDR conclusion may have been premature, as there is no conclusive data evidencing a correlation between a particular developmental time point that would render an individual most susceptible to toxicological insult with the development of autism. Therefore, it was our aim to determine if these chemicals could contribute to autistic like behaviors. We found that males treated with THMs and PCE have a significant reduction in the number of ultrasonic vocalizations (USVs) emitted in response to maternal separation, which are not attributed to deficits in vocal ability to or to lesser maternal care. These same males also show significantly elevated anxiety, an increase in perseverance behavior and a significant reduction in sociability. The sum of our data suggests that male, but not female mice, develop autistic like behaviors after gestational and postnatal exposure to the aforementioned chemical triad via drinking water. We believe development of such aberrant behaviors likely involves GABAergic system development.

Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene.
Carney E. W., Thorsrud B. A., Dugard P. H. and Zablotny C. L.
Birth Defects Res B Dev Reprod Toxicol. 2006;77(5):405-12.

The potential for trichloroethylene (TCE) and perchloroethylene (PERC) to induce developmental toxicity was investigated in Crl:CD (SD) rats whole-body exposed to target concentrations of 0, 50, 150 or 600 ppm TCE or 0, 75, 250 or 600 ppm PERC for six hours/day, seven days/week on gestation day (GD) 6-20 and 6-19, respectively. Actual chamber concentrations were essentially identical to target with the exception of the low PERC exposure level, which was 65 ppm. The highest exposure levels exceeded the limit concentration (2 mg/L) specified in the applicable test guidelines. Maternal necropsies were performed the day following the last exposure. Dams exposed to 600 ppm TCE exhibited maternal toxicity, as evidenced by decreased body weight gain (22% less than control) during GD 6-9. There were no maternal effects at 50 or 150 ppm TCE and no indications of developmental toxicity (including heart defects or other terata) at any exposure level tested. Therefore, the TCE NOEC for maternal toxicity was 150 ppm, whereas the embryo/fetal NOEC was 600 ppm. Maternal responses to PERC were limited to slight, but statistically significant reductions in body weight gain and feed consumption during the first 3 days of exposure to 600 ppm, resulting in a maternal NOEC of 250 ppm. Developmental effects at 600 ppm consisted of reduced gravid uterus, placental and fetal body weights, and decreased ossification of thoracic vertebral centra. Developmental effects at 250 ppm were of minimal toxicological significance, being limited to minor decreases in fetal and placental weight. There were no developmental effects at 65 ppm.

In vivo exposure of female rats to toxicants may affect oocyte quality.
Berger T. and Horner C. M.
Reprod Toxicol. 2003;17(3):273-81.

A potential endpoint for female reproductive toxicants is fertilizability of the oocytes. This endpoint has not been adequately examined for mammalian females. The objective of these studies was to evaluate fertilizability of rat oocytes following in vivo exposure to known male reproductive toxicants that exert effects via pathways that do not include endocrine disruption and to 4-vinylcyclohexene diepoxide, known to interfere with early follicular development. Oocytes were obtained from females following exposure and quality assessed by in vitro fertilization rate. One study evaluated fertilizability following 2 weeks exposure of females to inhaled tetrachloroethylene (2h/day, 5 days/week). The remaining studies evaluated fertilizability immediately following 2 weeks exposure via

drinking water to tetrachloroethylene, trichloroethylene, the fuel oxidants methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), tertiary amyl methyl ether (TAME), and a metabolite of the first two ethers 2-methyl-1,2-propanediol (2M2P), and to 4-vinylcyclohexene diepoxide. The percentage of oocytes fertilized was reduced following inhalation exposure to tetrachloroethylene, or consumption of trichloroethylene or TAME. Fertilizability was not altered by exposures to the other reproductive toxicants or to the other fuel oxidants. Consistent with the reduced oocyte fertilizability following exposure to trichloroethylene, oocytes from exposed females had a reduced ability to bind sperm plasma membrane proteins. Female reproductive capability assessed by the endpoint, oocyte fertilizability, was reduced by exposure to trichloroethylene and inhaled tetrachloroethylene.

A multidisciplinary approach to toxicological screening: II. Developmental toxicity.
Narotsky M. G. and Kavlock R. J.
J Toxicol Environ Health. 1995;45(2):145-71.

As part of the validation of an integrated bioassay for systemic toxicity, neurotoxicity, and developmental toxicity, we evaluated the effects of four pesticides, four chlorinated solvents, and two other industrial chemicals in Fischer 344 rats. The pesticides included carbaryl, triadimefon, chlordane, and heptachlor; the solvents included dichloromethane (DCM), carbon tetrachloride, trichloroethylene (TCE), and tetrachloroethylene (perchloroethylene, PER); and the industrial chemicals were di(2-ethylhexyl)phthalate (DEHP) and phenol. In the developmental toxicity studies, timed-pregnant rats were treated by gavage with vehicle or 1 of 2 dose levels of each compound on gestation d 6-19. The dams were allowed to deliver and their litters were examined on postnatal d 1, 3, and 6. Litter weights were determined on postnatal d 1 and 6. Implants were also counted to determine prenatal loss. Maternal toxicity was evidenced by dose-related alterations in weight gain for all 10 compounds. Clinical signs of maternal toxicity were present for all chemicals except chlordane and heptachlor. DEHP exposure resulted in the most pronounced developmental toxicity (high levels of pre- and postnatal mortality), whereas chlordane induced extensive postnatal loss. Of the solvents, only DCM did not cause a high incidence of full-litter resorption. Phenol, heptachlor, triadimefon, and carbaryl showed only slight potential for developmental toxicity. Malformations suggestive of teratogenicity included kinked tail (phenol), microphthalmia (TCE, PER, DEHP), and cleft palate with renal agenesis (DEHP). Although several findings (eye defects caused by TCE and PER, full-litter resorption and delayed parturition caused by PER, and delayed parturition/dystocia associated with triadimefon) have not been previously reported, the results are generally consistent with previous reports and

highlight the importance and relative ease of incorporation of developmental evaluations into a multidisciplinary screening battery.

B. Studies with unclear findings

A multidisciplinary approach to toxicological screening: IV. Comparison of results.
MacPhail R. C., Berman E., Elder J. A., Kavlock R. J., Moser V. C., Narotsky M. G. and Schlicht M.
J Toxicol Environ Health. 1995;45(2):211-20.

Toxicity data collected under standardized test conditions may be of the utmost importance in health risk assessment, in which human exposure limits are often derived from laboratory experiments. A standardized approach to data collection is also important for evaluating the sensitivity and specificity of test methods used to determine toxic potential. Several experiments were undertaken to determine the effects of chemical exposures using a multidisciplinary screening battery, which included tests for systemic, neurological and developmental toxicity. The effects of 1- and 14-d exposures to 10 chemicals on systemic and neurological indices of toxicity were determined in female F344 rats using standardized test batteries. Parallel experiments determined chemical effects on prenatal and postnatal development following exposure of the dams for 14 d. The chemicals included four pesticides (carbaryl, triadimefon, chlordane, and heptachlor), four solvents (trichloroethylene, tetrachloroethylene, carbon tetrachloride, and dichloromethane), and two industrial compounds (phenol and diethylhexyl phthalate). The results showed that the chemicals produced markedly different qualitative patterns of effect on systemic, neurological, and developmental indices of toxicity. Differences in the pattern of systemic and neurological effects were also obtained that depended on dosing duration. Quantitative analyses indicated that the highest ineffective dose as well as the lowest effective dose could vary by as much as two orders of magnitude across the different indices of toxicity. These results clearly show that a test battery focused on a single endpoint of toxicity cannot be used to accurately predict either qualitatively or quantitatively a chemical's systemic, neurological, and developmental toxicity profile.

C. Related articles

A mixture of environmental contaminants increases cAMP-dependent protein kinase in Spisula embryos.
Kreiling J. A., Stephens R. E. and Reinisch C. L.
Environmental Toxicology and Pharmacology. 2005;19(1):9-18.

Using the surf clam embryo, we investigated the effects of the combination of bromoform, chloroform, and tetrachloroethylene, three pollutants found in high concentrations in the municipal water supply in Brick, New Jersey. Exposure produced an increase in an isoform of the regulatory subunit (RII) of cAMP-dependent protein kinase, demonstrated by confocal microscopy and western blotting. Embryos showed an increase in RII where the primordial gill and ciliated velar epithelium are innervated. This increase correlated with increased ciliary activity, indicating a corresponding rise in the catalytic subunit. Treatment resulted in decreased threonine phosphorylation of actin. There was no effect on neurotransmitters or receptors of the serotonergic-dopaminergic nervous system. These effects occurred only with the ternary mixture. No significant effect was seen with individual or paired components. This is the first report showing that bromoform, chloroform, and tetrachloroethylene act synergistically to alter a key regulator of neuronal development.

Volatile organic compounds inhibit human and rat neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes.
Bale A. S., Meacham C. A., Benignus V. A., Bushnell P. J. and Shafer T. J.
Toxicol Appl Pharmacol. 2005;205(1):77-88.

The relative sensitivity of rats and humans to volatile organic compounds (VOCs) such as toluene (TOL) and perchloroethylene (PERC) is unknown and adds to uncertainty in assessing risks for human exposures to VOCs. Recent studies have suggested that ion channels, including nicotinic acetylcholine receptors (nAChRs), are targets of TOL effects. However, studies comparing TOL effects on human and rat ligand-gated ion channels have not been conducted. To examine potential toxicodynamic differences between these species, the sensitivity of human and rat nAChRs to TOL was assessed. Since PERC has similar effects, in vivo, to TOL, effects of PERC on nAChR function were also examined. Two-electrode voltage-clamp techniques were utilized to measure acetylcholine-induced currents in neuronal nAChRs (alpha4beta2, alpha3beta2, and alpha7) expressed in Xenopus oocytes. PERC (0.065 mM) inhibited alpha7 nAChR currents by 60.1 +/- 4.0% (human, n = 7) and 40 +/- 3.5% (rat, n = 5), and inhibited

alpha4beta2 nAChR currents by 42.0 +/- 5.2% (human, n = 6) and 52.2 +/- 5.5% (rat, n = 8). Likewise, alpha3beta2 nAChRs were significantly inhibited by 62.2 +/- 3.8% (human, n = 7) and 62.4 +/- 4.3% (rat, n = 8) in the presence of 0.065 mM PERC. TOL also inhibited both rat and human alpha7, alpha4beta2, and alpha3beta2 nAChRs. Statistical analysis indicated that although there was not a species (human vs. rat) difference with PERC (0.0015-0.065 mM) or TOL (0.03-0.9 mM) inhibition of alpha7, alpha4beta2, or alpha3beta2 nAChRs, all receptor types were more sensitive to PERC than TOL. These results demonstrate that human and rat nACh receptors represent a sensitive target for VOCs. This toxicodynamic information will help decrease the uncertainty associated with animal to human extrapolations in the risk assessment of VOCs.

Effects of peroxisome proliferators in the medaka embryo-larval assay.
Haasch M. L.
Faseb J. 2003;17(4 Pt 1).

The Japanese medaka (Oryzias latipes) was used in the medaka embryo larval assay (MELA) to determine possible adverse developmental effects of peroxisome proliferators (PPs). PPs include hypolipidemia pharmaceuticals (e.g., clofibrate; CLO) and environmental contaminant-like plasticizers (e.g., dibutylphthalate; DBP), solvents (e.g. tetrachloroethylene; PCE) and herbicides (e.g., 2,4-D). Peroxisome proliferation occurs in a species-specific manner. The medaka is resistant to peroxisome proliferation but it is not known if adult sensitivity is predictive of developmental sensitivity. MELA were performed with DBP, PCE, 2,4-D, WY14643, and CLO at concentrations up to 10 ppm. DBP at 10 ppm (35.9 nM) was lethal and 1 ppm produced severe malformations including tube heart, blood clot and failure to form the circulatory system. Effects of PCE at 10 ppm (60.3 nM) were not significant but included delayed growth, skeletal and circulatory defects. 2,4-D was lethal at 1 ppm (4.52 nM) during day 1 of development. Wy14643 was not lethal up to 10 ppm (30.9 nM) but CLO, a less potent PP, was lethal at 10 ppm (41.2 nM). Taken together these data indicate that adult susceptibility to peroxisome proliferator may not be predictive of developmental sensitivity and exposure to different PPs produces different adverse developmental effects that may not be related to PP potency.

Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation.

Gentry P. R., Covington T. R. and Clewell H. J., 3rd
Regulatory toxicology and pharmacology : RTP. 2003;38(1):1-16.

In recent years efforts have increased to develop a framework for assessing differences, both pharmacokinetic and pharmacodynamic, between children and adults for purposes of assessing risk of adverse effects following chemical exposure. The specific goal of this study was to demonstrate an approach for using PBPK modeling to compare maternal and fetal/neonatal blood and tissue dose metrics during pregnancy and lactation. Six chemical classes were targeted to provide a variety of physicochemical properties (volatility, lipophilicity, water solubility), and surrogate chemicals were selected to represent each class (isopropanol, vinyl chloride, methylene chloride, perchloroethylene, nicotine, and TCDD), based on the availability of pharmacokinetic information. These chemicals were also selected to provide different pharmacokinetic characteristics, including metabolic production of stable or reactive intermediates in the liver and competing pathways for metabolism. Changes in dosimetry during pregnancy predicted by the modeling were mainly attributable to the development of enzymatic pathways in the fetus or to changes in tissue composition in the mother and fetus during pregnancy. In general, blood concentrations were lower in the neonate during the lactation period than in the fetus during gestation. This postnatal decrease varied from only a slight change (for TCDD) to approximately four orders of magnitude (for vinyl chloride). As compared to maternal exposure, fetal/neonatal exposures ranged from approximately twice as great (for TCDD) to several orders of magnitude lower (for isopropanol). The results of this study are in general agreement with the analyses of data on pharmaceutical chemicals, which have suggested that the largest difference in pharmacokinetics observed between children and adults is for the perinatal period. The most important factor appears to be the potential for decreased clearance of toxic chemicals in the perinatal period due to immature metabolic enzyme systems, although this same factor can also reduce the risk from reactive metabolites during the same period.

Effects of tetrachloroethylene on the viability and development of embryos of the Japanese medaka, Oryzias latipes.
Spencer H. B., Hussein W. R. and Tchounwou P. B.
Arch Environ Contam Toxicol. 2002;42(4):463-9.

We evaluated the acute toxicity of Tetrachloroethylene (C(2)Cl(4)), and investigated its sub-chronic effects on the embryonic development of Japanese medaka (Oryzias latipes). One-day-old eggs/embryos of this fish species were exposed, under static renewal conditions, to serial concentrations (0, 20, 40, 60, and 80 mg/L) of C(2)Cl(4) for 96 h (acute) and 10 days (sub-chronic) time periods. The toxic endpoints evaluated included: egg/embryo viability, hatchability, and morphological/developmental abnormalities. The acute toxicity test resulted in a 96 h-LC(50) of 27.0 (19.5-32.9) mg/L for egg viability. Exposure of eggs to sub-chronic concentrations (0, 1.5, 3, 6, 12, and 25 mg/L) of C(2)Cl(4) significantly reduced hatchability and larval survival, in a concentration dependent manner. At the highest tested concentration (25 mg/L) of the sub-lethal exposure, larval survival was greatly reduced to within three days post-hatch. The lowest tested concentration (1.5 mg/L) produced a significant number of developmental effects to the Japanese medaka, including abnormal development of the circulatory system, yolk-sac edema, pericardial edema, scoliosis, hemorrhaging, blood pooling, and defects in heart morphology. The severity of these abnormalities was concentration-dependent. It can be concluded from these results that tetrachloroethylene is teratogenic to the Japanese medaka.

Developmental toxicity of trichloroethylene, tetrachloroethylene and four of their metabolites in rat whole embryo culture.
Saillenfait A. M., Langonne I. and Sabate J. P.
Arch Toxicol. 1995;70(2):71-82.

The embryotoxicity of trichloroethylene (TRI), tetrachloroethylene (PER), and of four of their oxidative metabolites i.e. trichloroacetic acid, dichloroacetic acid, chloral hydrate, and trichloroacetyl chloride, was studied in vitro, using the rat whole embryo culture system. Embryos from Sprague-Dawley rats were explanted on gestational day 10 (plug day = day 0) and cultured for 46 h in the presence of the test chemical. All of the tested chemicals produced concentration-dependent decreases in growth and differentiation and increases in the incidence of morphologically abnormal embryos. TRI and PER produced qualitatively similar patterns of abnormalities, while TRI and/or PER metabolites, each elicited clearly distinguishable dysmorphogenic profiles. The

presence of hepatic microsomal fractions in the culture medium produced marked decreases in TRI- and PER-induced embryotoxic effects, including mortality, severity of malformations, and delayed growth and differentiation.

Brain lipid and fatty acid composition of the cerebral cortex of guinea pig pups after intrauterine exposure to perchloroethylene.
Kyrklund T. and Haglid K. G.
J Neurochem. 1989;52(Suppl).

Pregnant guinea pig dams were continuously exposed to perchloroethylene (160 ppm) during the last half of gestation (days 33-65). The gross lipid composition and the fatty acid pattern of ethanolamine phosphoglyceride was determined in the cerebral cortex of the pups. A small decrease in stearic acid was observed in the pups exposed to perchloroethylene. A similar change has previously been observed in adult rats exposed to perchloroethylene. It thus seems that intrauterine exposure during the period of rapid brain growth does not result in greater changes than those known to appear in brain lipids of adult animals exposed to perchloroethylene.

Trichloroacetic acid accumulates in murine amniotic fluid after tri- and tetrachlororoethylene inhalation.
Ghantous H., Danielsson B. G., Dencker L., Gorczak J. and Vesterberg O.
Acta Pharmacol Toxicol (Copenh). 1986;58(2):105-14.

The distribution of trichloroethylene (Tri) and tetrachloroethylene (Tetra) and their metabolites have been studied in pregnant mice by means of whole-body autoradiography (14C-labelled Tri and Tetra) and gas chromatography, with special emphasis on possible uptake and retention in the foetoplacental unit. Volatile (non-metabolized) activity appeared at short intervals after a 10 min. or 1 hr inhalation period in foetus and amniotic fluid. Most notable, however, was a strong accumulation and retention (peak at 4 hrs) in amniotic fluid of the metabolite trichloroacetic acid (TCA) after inhalation of either of the solvents. The main metabolite of Tri, trichloroethanol (TCE) (or conjugates), did not accumulate specifically as compared to maternal plasma. TCA infused intravenously in the maternal plasma was accumulated in amniotic fluid, but less pronounced than after Tri and Tetra inhalation, indicating that some metabolism of Tri and Tetra to TCA may occur in the foetoplacental unit. The results suggest that TCA may be transported to the foetus partly paraplacentally through foetal membranes and amniotic fluid, with the possibility of foetal swallowing or absorption through the skin. Foetal urinary activity also suggests that circulation between foetus and amniotic fluid may contribute to the long-term retention in the foetoplacental unit. In the mother,

after inhalation exposures, and in intraperitoneally injected newborn mice, non-extractable radioactivity was found in the respiratory tract, liver, and kidney, indicating binding to these organs through metabolism.

BRINE SHRIMP (ARTEMIA SALINA) NAUPLII AS A TERATOGEN TEST SYSTEM.
Kerster H. W. and Schaeffer D. J.
Ecotoxicol Environ Saf. 1983;7(3):342-49.

Brine shrimp increase in length rapidly after hatching. A teratogen test system is based on disruption of elongation between 24 and 48 hr after wetting of the cysts. Teratogenicity of substances dissolved in the medium is assayed by comparison of average lengths of animals raised for the test period in suspect solution with average lengths of controls. The system is fast, inexpensive, and requires little skill. Brine shrimp are suited to testing industrial wastes, chemical formulations, drugs, and food additives that can be dissolved in water at 25 degrees C. The method appears unsuited to testing the teratogenicity of gases, particulates, very dilute wastes, or natural waters. Cadmium, mercury, lead, zinc, bromoform, n-butylphthalate, 1,2-dichloroethane, nitrobenzene, tetrachloroethylene, toluene, 1,2,4-trichlorobenzene, and 1,1,3-trichloroethane were found teratogenic. Chromium (III), chromium (VI), copper, chlorobenzene, chloroform, dimethyl sulfoxide (DMSO), and phenol were found not teratogenic. Other aquatic organism teratogen test systems are surveyed.

Effects of methylene chloride, trichloroethane, trichloroethylene, tetrachloroethylene and toluene on the development of chick embryos.
Elovaara E., Hemminki K. and Vainio H.
Toxicology. 1979;12(2):111-9.

Toluene and 5 aliphatic chlorinated hydrocarbons of wide industrial use were injected into the air space of fertilized chicken eggs at 2, 3 and 6 days of incubation. The embryotoxicity was evaluated as survival and death incidences after 14 days of incubation, and also the weights and lengths of the embryos were recorded. The approximate LD50 value for trichloroethylene and trichloroethanes varied between 50 and 100 mumol/egg while for toluene, tetrachloroethylene and methylene chloride it was over 100 mumol/egg. Macroscopic malformations of various kinds were produced with doses of 5-100 mumol/egg. The teratogenic potential of the tested compounds decreased in the following order: 1,1,1-trichloroethane greater than trichloroethylene greater than methylene chloride, tetrachloroethylene, 1,1,2-trichloroethane greater than toluene greater than olive oil control.

D. Titles only (abstracts not available)

Biological actions and interactions of tetrachloroethylene.
Reichert D.
Mutat Res. 1983;123(3):411-29.

TERATOLOGY STUDIES IN MICE EXPOSED TO MUNICIPAL DRINKING-WATER CONCENTRATES DURING ORGANOGENESIS.
Kavlock R., Chernoff N., Carver B. and Kopfler F.
Food Cosmet Toxicol. 1979;17(4):343-47.

Behavioral teratology of perchloroethylene in rats.
Nelson B. K., Taylor B. J., Setzer J. V. and Hornung R. W.
J Environ Pathol Toxicol. 1979;3(1-2):233-50.

The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats.
Schwetz B. A., Leong K. J. and Gehring P. J.
Toxicol Appl Pharmacol. 1975;32(1):84-96.