NO SIGNFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN TETRANITROMETHANE

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SUMMARY OF FINDINGS

The carcinogenic potency of tetranitromethane was estimated from dose-response data for alveolar/bronchiolar carcinomas or adenomas combined in male $B6C3F_1$ mice reported by the National Toxicology Program (NTP, 1990). The cancer potency estimate corresponds to the upper 95 percent confidence bound on the linear term of the multistage model fit to cancer dose-response data in animals.

Using the default procedures outlined in Title 22 (California Code of Regulations, Section 12703), the Office of Environmental Health Hazard Assessment (OEHHA) derived a human cancer potency estimate of 12 $(mg/kg\text{-}day)^{-1}$ for tetranitromethane. The potency derivation takes into account body size differences between humans and experimental animals. The Proposition 65 "no significant risk level" (NSRL) is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. Based on this potency estimate, the intake level associated with a lifetime cancer risk of 10^{-5} is 0.059 µg/day. Cancer potency estimates and the corresponding NSRL are given in Table 1.

Table 1. Human Cancer Potency and NSRL for Tetranitromethane.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (µg/day)
Tetranitromethane	12	0.059

INTRODUCTION

This report describes the derivation of a cancer potency value and no significant risk level (NSRL) for tetranitromethane (CAS number 509-14-8, molecular weight 196.04). "Tetranitromethane" was listed on July 1, 1990 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). Tetranitromethane is the principal volatile contaminant of tetranitrotoluene (TNT). Tetranitromethane has been used in explosives, rocket propellants, as an additive to increase the cetane number of diesel fuel and as a

chemical reagent (NTP, 1990). Tetranitromethane is not known to occur naturally (IARC, 1996).

This document discusses the studies available for cancer dose response assessment, and summarizes the derivations of the cancer potency estimate and NSRL. A description of the methodology used is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

The carcinogenicity of tetranitromethane was investigated by NTP in studies in which tetranitromethane was administered to male and female rats and mice by inhalation (NTP, 1990). These are the only studies available for estimating the potency of tetranitromethane.

The dose-response data for the NTP studies in male and female F344/N rats are presented in Table 2. Groups of 50 male and 50 female F344/N rats were exposed via inhalation to tetranitromethane six hours per day, five days per week at 0, 2 or 5 ppm for 103 weeks. The survival of the high dose male rats was significantly lower than that of the controls after day 590. No other statistically significant differences in survival were seen between any groups of either sex. NTP observed significant increases in the incidences of alveolar and bronchiolar carcinomas and adenomas and squamous cell carcinomas of the lung with increasing dose in both sexes (NTP, 1990).

Table 2. Lung Neoplasms in Rats Treated with Tetranitromethane via Inhalation for 103 Weeks (NTP, 1990).

Sex, Strain	Chamber Concentration (ppm)	Lifetime Average Dose[1] (mg/kg-day)	Alveolar/Bronchiolar Carcinomas/ Adenomas[2]	Squamous Cell Carcinomas of the Lung[2]
Male F344/N	0	0	1/45	0/50
	2	1.691	33/48[3]	1/50
	5	4.227	46/50[3]	19/46[3]
Female F344/N	0	0	0/48	0/45
	2	1.984	22/49[3]	1/49
	5	4.960	50/50[3]	12/47[3]

[1] Tetranitromethane was administered six hours per day, five days per week, for 103 weeks. Lifetime average dose was calculated as described in the Appendix.
[2] Number of tumor-bearing animals/number of animals alive at first occurrence of tumor in any of the three groups. The first alveolar/bronchiolar adenoma/carcinoma occurred in week 63 for males and week 51 for females. The first squamous cell carcinoma of the lung occurred in week 74 for males and week 73 for females.
[3] Statistical significance ($p<0.001$) using pairwise comparison (Fisher Exact Test).

The dose-response data for the NTP studies in male and female $B6C3F_1$ mice are presented in Table 3. Groups of 50 male and 50 female $B6C3F_1$ mice were exposed via inhalation to

tetranitromethane six hours per day, five days per week at 0, 0.5 or 2 ppm for 103 weeks. The survival of the low dose male mice was significantly lower than that of the controls after day 684, and the survival of the high dose male mice was significantly lower than that of controls after day 546. The survival of both dose groups of female mice was comparable to that of controls. NTP observed significant increases in the incidences of alveolar and bronchiolar carcinomas and adenomas with increasing dose in both sexes.

Table 3. Alveolar and Bronchiolar Carcinomas and Adenomas in Mice Treated with Tetranitromethane via Inhalation for 103 Weeks (NTP, 1990).

Sex, Strain	Chamber Concentration (ppm)	Lifetime Average Dose[1] (mg/kg-day)	Tumor Incidence[2]	Statistical Significance[3]
Male	0	0	12/50	--
$B6C3F_1$	0.5	0.937	27/47	$p < 0.001$
	2	3.748	47/48	$p < 0.001$
Female	0	0	4/47	--
$B6C3F_1$	0.5	0.981	24/46	$p < 0.001$
	2	3.924	49/49	$p < 0.001$

[1] Tetranitromethane was administered six hours per day, five days per week, for 103 weeks. Lifetime average dose was calculated as described in the Appendix.
[2] Number of tumor-bearing animals/number of animals alive at first occurrence of tumor in any of the three groups. The first alveolar/bronchiolar adenoma/carcinoma occurred in males in week 54 and in females in week 63.
[3] Results of pairwise comparison using the Fisher Exact Test.

APPROACH TO DOSE RESPONSE ANALYSIS

Tetranitromethane was tested for mutagenic activity in the *Salmonella*/mammalian microsome assay. Tetranitromethane showed strong genotoxic activity: it was mutagenic in all tester strains used (TA97, TA98, TA100, and TA102). The mutagenicity was independent of an *in vitro* metabolic activation system (Wurgler *et al*., 1990). A study examining mutations of the K-*ras* oncogene in rat lung tumors showed that all lung tumors (19/19) induced by tetranitromethane contained mutations of K-*ras* at codon 12, compared to no mutations at this site in lung tumors arising in sham-exposed animals (Belinsky *et al.*, 1997). Tetranitromethane is a potent protein nitrating agent and has been proposed to have a role in the deamination of DNA (Belinsky *et al.*, 1997, citing Stowers *et al.*, 1987). In the case of the mutations of the K-*ras* gene, the authors of the study proposed that the specific mutation observed could have resulted from the deamination of cytosine resulting in base misrepair. NTP has also reported that tetranitromethane increased sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells (NTP, 1990).

These findings strongly suggest a genotoxic mode of action. There is insufficient information on the precise mechanism of carcinogenicity to permit the development of a biologically based

model for cancer potency estimation. There are also insufficient data to support dose adjustments based on pharmacokinetic models. Therefore, the default approach (i.e., a linearized multistage model and interspecies scaling) has been applied. The approach used is described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

Cancer potency estimates were derived from the studies described above (Table 4). NTP observed significant increases in two types of lung neoplasms in male and female rats (alveolar/bronchiolar carcinomas/adenomas and squamous cell carcinomas of the lung); therefore, potencies were calculated for both tumor types, along with the adjusted (human equivalent) potencies. Potency estimates for the squamous cell carcinoma incidence were low and did not contribute significantly to the overall cancer risk in the rat.

Table 4: Animal and Human Potency Estimates for Tetranitromethane.

Sex and Species	Type of Neoplasm	Animal Cancer Potency $(mg/kg\text{-}d)^{-1}$	Human Cancer Potency $(mg/kg\text{-}d)^{-1}$
Male F344/N rat	Alveolar/Bronchiolar Carcinomas/Adenomas	0.788	4.3
	Squamous Cell Carcinoma of the Lung	0.004	0.02
Female F344/N rat	Alveolar/Bronchiolar Carcinomas/Adenomas	0.788	5.1
	Squamous Cell Carcinoma of the Lung	0.004	0.03
Male $B6C3F_1$ mouse	Alveolar/Bronchiolar Carcinomas/Adenomas	0.975	12
Female $B6C3F_1$ mouse	Alveolar/Bronchiolar Carcinomas/Adenomas	0.808	10

Bolding indicates values selected as the basis of the NSRL.

The male mouse was found to be the most sensitive species/sex tested by the NTP in its carcinogenicity studies of tetranitromethane. A cancer potency estimate of 12 $(mg/kg\text{-}d)^{-1}$, which includes adjustments for rodent-human differences in body size, was obtained from these data.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The cancer potency estimate derived above from studies in male mice was used to calculate the NSRL of 0.059 µg/day for tetranitromethane.

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR TETRANITROMETHANE

Procedures for the development of Proposition 65 NSRLs are described in regulation (California Code of Regulations, Title 22, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRL for Tetranitromethane is outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

"Multistage" polynomial

For regulatory purposes, the lifetime probability of dying with a tumor (p) induced by an average daily dose (d) is often assumed to be (CDHS, 1985; U.S. EPA, 1996; Anderson *et al.*, 1983):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)] \quad (1)$$

with constraints,

$$q_i \geq 0 \text{ for all i.}$$

The q_i are parameters of the model, which are taken to be constants and are estimated from the data. The parameter q_0 represents the background lifetime incidence of the tumor. The parameter q_1, or some upper bound, is often called the cancer potency, since for small doses it is the ratio of excess lifetime cancer risk to the average daily dose received. For the present discussion, cancer potency will be defined as q_1^*, the upper 95% confidence bound on q_1 (CDHS, 1985), estimated by maximum likelihood techniques. When dose is expressed in units of mg/kg-day, the parameters q_1 and q_1^* are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977). To estimate potency in animals (q_{animal}) from experiments of duration T_e, rather than the natural life span of the animals (T), it is assumed that the lifetime incidence of cancer increases with the third power of age:

$$q_{animal} = q_1^* \bullet (T/T_e)^3 \quad (2)$$

Following Gold and Zeiger (1997) and the U.S. Environmental Protection Agency (U.S. EPA, 1988), the natural life span of mice and rats is assumed to be two years, so that for experiments lasting T_e weeks in these rodents:

$$q_{animal} = q_1^* \bullet (104/T_e)^3 \quad (3)$$

To estimate risk at low doses, potency is multiplied by average daily dose. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Calculation of the lifetime average dose

The lifetime average dose in units of mg/kg-day was calculated for each of the relevant dose groups, based on the dose level, duration and regimen described in the experiments above. When actual body weight information was not provided by the study authors, default values were utilized as described by Gold and Zeiger (1997). In this case, for tetranitromethane, body weights for male and female mice (0.039 kg and 0.034 kg, respectively) and male and female rats (0.425 kg and 0.263 kg, respectively) were taken from data in the NTP report (NTP, 1990). The inhalation rates for rats and mice were calculated following the method described by

Anderson (Anderson, 1983). Briefly, the air concentration of tetranitromethane in units of ppm was converted to units of mg/m^3 by multiplying by 8 (mg/m^3)/ppm. This number was then multiplied by the inhalation rate for mice or rats and divided by the body weight (given above); multiplied by 6/24 to account for the six hour per day exposure, multiplied by 5/7 to account for a five day per week dosing and adjusted for less than lifetime dosing by multiplying by 103/104 weeks. The resulting inhalation rates were 0.254 m^3/day for male rats, 0.184 m^3/day for female rats; and 0.052 m^3/day for male mice, 0.047 m^3/day for female mice.

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, human potency is estimated. As described in the California risk assessment guidelines (CDHS, 1985), a dose in units of milligram per unit surface area is assumed to produce the same degree of effect in different species in the absence of information indicating otherwise. Under this assumption, scaling to the estimated human potency (q_{human}) can be achieved by multiplying the animal potency (q_{animal}) by the ratio of human to animal body weights (bw_h/bw_a) raised to the one-third power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$:

$$q_{human} = q_{animal} \bullet (bw_h / bw_a)^{1/3} \tag{4}$$

A.3 Risk-Specific Intake Level Calculation

The intake level (I, in mg/day) associated with a cancer risk R, from exposure is:

$$I = \frac{R \times bw_h}{q_{human}} \tag{5}$$

where bw_h is the body weight, and q_{human} the theoretical cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the no significant risk level for cancer under Proposition 65 (Title 22 California Code of Regulations, Section 12703). Thus for a 70 kg person, the NSRL is given by:

$$NSRL = \frac{10^{-5} \times 70\,kg}{q_{human}} \tag{6}$$

APPENDIX REFERENCES

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