NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN BENZOFURAN

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Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
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SUMMARY OF FINDINGS

The cancer potency of benzofuran was estimated from dose-response data for multiple benzofuran-responding tumor sites observed among male mice exposed orally (NTP, 1989). These sites were liver, forestomach and lung. For each of the tumor sites listed above, a probability distribution of cancer potency estimates was derived using likelihood theory. The linear term (q_1) of the multistage model fit to dose response data for a given site represents the cancer potency for that site. The cancer potencies for the affected sites were summed probabilistically, according to their distributions, to obtain a combined distribution. This combined distribution representing cancer potency for all selected sites affected by benzofuran was derived through Monte Carlo analysis. The upper 95 percent confidence bound, indicated by the combined distribution for these benzofuran-related tumor sites, was taken as the cancer potency for benzofuran.

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. The cancer potency estimate and corresponding NSRL are given in Table 1.

Table 1. Cancer Potency and NSRL for Benzofuran.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (µg/day)
Benzofuran	0.63	1.1

INTRODUCTION

This report describes the derivation of a cancer potency value and NSRL for benzofuran (CAS number 271-89-6, molecular weight 118.1). "Benzofuran" was listed on October 1, 1990 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). Benzofuran is used commercially as a chemical intermediate in the manufacturing of resins used in paintings, varnishes and other coatings for a variety of uses (NTP, 1989; IARC, 1995). Benzofuran is a constituent of coal tar and is released into the

environment from activities related to coke production, coal gasification and oil-shale processing (IARC, 1995).

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 benzofuran has been investigated in gavage studies in rats and mice (NTP, 1989). NTP (1989) described a previous 12-month study by Stankevich (1962), in which benzofuran was administered via subcutaneous implantation to albino rats (strain not specified). Rats exhibited an incidence of 8.5% of subcutaneous fibromas; no data for controls were reported. The study by Stankevich (1962) is not suitable for potency estimation.

In the NTP (1989) studies, rats and mice (50/sex/dose group) were administered benzofuran in corn oil by gavage, five days per week for 104 weeks. Daily doses administered to rats were 0, 30 or 60 mg/kg for males and 0, 60 or 120 mg/kg for females. Daily doses administered to mice were 0, 60 or 120 mg/kg for males and 0, 120 or 240 mg/kg for females. No treatment-related tumors were observed among male rats. Female rats exhibited a low, but statistically significant, increase in tubular cell adenocarcinomas of the kidney. Incidences of female rat kidney tumors were 0/50, 1/50 and 4/50 for the 0, 60 and 120 mg/kg dose groups, respectively.

Among male mice, increased incidences of various tumors of the liver, forestomach and lung were observed relative to controls (NTP, 1989). Tumor incidences in male mice are shown in Table 2. Among female mice, increased incidences of tumors of the liver, lung and forestomach were also observed relative to controls (NTP, 1989). Tumor incidences of female mice are shown in Table 3.

Survival of benzofuran-exposed female rats and male mice was similar to survival among controls. Survival of benzofuran-exposed male rats was reduced relative to controls after 92 weeks. Survival of benzofuran-exposed female rats was reduced relative to controls after 89 weeks. Mean body weights of male rats (high-dose group) and female rats (high-dose group) and male mice (both dose groups) were 4 to 11 percent lower than body weights of controls. Mean body weights of benzofuran-treated female mice were 8 to 35 percent lower than those of vehicle controls.

Table 2. Tumor Incidence[a] in Male B6C3F$_1$ Mice Receiving Benzofuran Via Gavage for 104 Weeks (NTP, 1989).

Tumor Site and Type		Lifetime Average Daily Dose (mg/kg-day)[b]			Trend[d]
		0	42.9	85.7	
Liver	Hepatocellular adenoma	4/48	24/35[c]	34/43[c]	p<0.0001
	Hepatocellular adenoma or carcinoma (combined)	12/48	30/35[c]	37/43[c]	p<0.0001
	Hepatoblastoma	0/48	3/35	18/43[c]	p<0.0001
	Hepatocellular adenoma, carcinoma, or hepatoblastoma	12/48	31/35[c]	40/43[c]	p<0.0001
Forestomach	Squamous-cell papilloma	2/49	7/39[c]	10/48[c]	p=0.007
	Squamous-cell papilloma or carcinoma	2/49	11/39[c]	13/48[c]	p=0.0012
Lung	Alveolar/bronchiolar adenoma	4/48	7/35	15/45[c]	p=0.057
	Alveolar/bronchiolar adenoma or carcinoma	10/48	9/35	19/45[c]	p=0.02

[a] Effective tumor rates were calculated from the individual animal data provided in the NTP Technical Report (NTP, 1989) and represent the number of tumor-bearing mice among the number of mice alive at the appearance of the first tumor in either the control or treated groups.

[b] Lifetime average daily doses were estimated by adjusting the administered doses (0, 60, 120 mg/kg-d) by a factor of 5/7 to account for dosing five days per week.

[c] Significantly increased relative to the control group, $p<0.05$ (Fisher Exact Test).

[d] Exact trend test.

Table 3. Tumor Incidence[a] in Female B6C3F$_1$ Mice Receiving Benzofuran Via Gavage for 104 Weeks (NTP, 1989).

Tumor Site and Type		Lifetime Average Daily Dose (mg/kg-day)[b]			
		0	42.9	85.7	Trend[d]
Liver	Hepatocellular adenoma	1/46	22/44[c]	21/44[c]	p<0.0001
	Hepatocellular adenoma or carcinoma (combined)	4/46	25/44[c]	22/44[c]	p<0.0001
Forestomach	Squamous-cell papilloma	2/46	8/41[c]	5/43	p=0.17
	Squamous-cell papilloma or carcinoma	2/46	9/41[c]	5/43	p=0.18
Lung	Alveolar/bronchiolar adenoma	1/44	5/37	13/39[c]	p<0.0001
	Alveolar/bronchiolar adenoma or carcinoma	2/44	9/37[c]	14/39[c]	p<0.0001

[a] Effective tumor rates were calculated from the individual animal data provided in the NTP Technical Report (NTP, 1989) and represent the number of tumor-bearing mice among the number of mice alive at the appearance of the first tumor in either the control or treated groups.

[b] Lifetime average daily doses were estimated by adjusting the administered doses (0, 120, 240 mg/kg-d) by a factor of 5/7 to account for dosing five days per week.

[c] Significantly increased relative to the control group, p<0.05 (Fisher Exact Test)

[d] Exact trend test

APPROACH TO DOSE-RESPONSE ANALYSIS

Benzofuran was not observed to induce mutations in bacterial test systems, but did induce gene mutations and chromosomal damage in mammalian cells (reviewed in IARC, 1995). Very little is known about the potential mechanism of carcinogenicity for benzofuran (IARC, 1995), however, in light of the genotoxicity data, a genotoxic mode of action is presumed. There are insufficient data to support dose adjustments based on pharmacokinetic models (IARC, 1995). 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. Additionally, since benzofuran induced tumors at multiple sites in male mice (the most sensitive sex and species), a combined cancer potency estimate was derived for benzofuran-treatment related cancer sites judged likely to contribute to the overall cancer potency using Monte Carlo analysis (see below).

DOSE RESPONSE ASSESSMENT

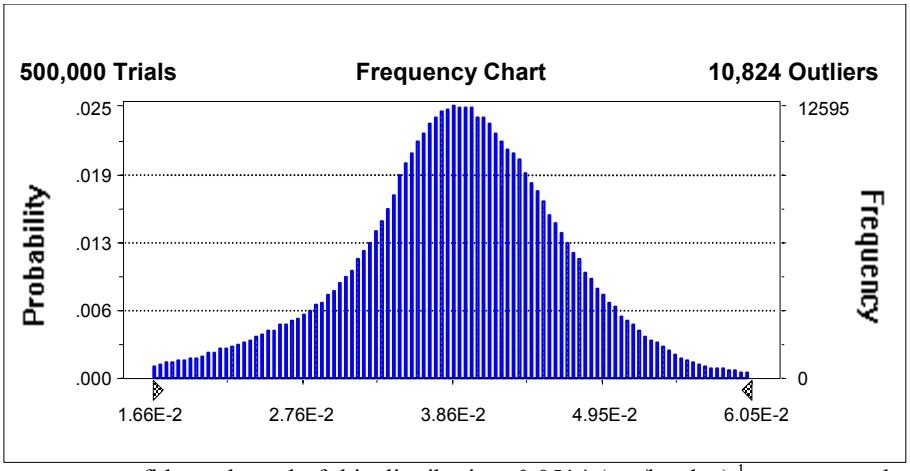
Cancer potency estimates were derived from sensitive tumor responses from the studies, as evident by trend tests (Tables 2 and 3) (NTP, 1989). Among male mice, these include liver

adenoma, liver adenoma/carcinoma (combined), hepatoblastoma, liver adenoma/carcinoma/-hepatoblastoma (combined), forestomach papilloma, forestomach papilloma/carcinoma (combined), lung adenoma, and lung adenoma/carcinoma (combined) (Table 4). Among female mice, these include liver adenoma, liver adenoma/carcinoma (combined), lung adenoma, and lung adenoma/carcinoma (combined) (Table 4).

Since benzofuran induced tumors at multiple sites in mice, a combined potency estimate for all treatment-related tumor sites among male mice (the most sensitive sex) was derived using Monte Carlo analysis. For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model was generated with the MSTAGE computer program (Crouch, 1998), which had been modified to tabulate percentile values. A combined distribution (Figure 1) was created by adding q_1 for each tumor site, according to its distribution, through 500,000 Monte Carlo trial simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound of the combined distribution was taken as the basis of the cancer potency estimate for the combined tumor sites (Table 4).

Distributions of the cancer potency estimates were combined for the following sites: liver adenoma, carcinoma, or hepatoblastoma (combined), forestomach papilloma or carcinoma (combined), and lung adenoma or carcinoma (combined) among male mice (Figure 1).

Figure 1. Combined Distribution of Potency Estimates for All Benzofuran-Related Tumor Sites Among Male Mice.



[1] The 95 percent upper confidence bound of this distribution, 0.0514 $(mg/kg\text{-}day)^{-1}$, represents the lifetime animal cancer potency for all treatment-related tumors in male mice. Using methods described in the Appendix, this estimate was scaled to a human potency estimate of 0.63 $(mg/kg\text{-}day)^{-1}$.

Table 4. Human Cancer Potency Estimates for Selected Benzofuran-Induced Tumors.

Tumor Site	Cancer Potency Estimate $(mg/kg\text{-}day)^{-1}$	
	Males	**Females**
Liver adenoma	0.31	0.086
Liver adenoma or carcinoma	0.41	0.088
Hepatoblastoma	0.051	---
Liver adenoma, carcinoma, or hepatoblastoma[1]	0.55	---
Forestomach papilloma	0.051	NE[2]
Forestomach papilloma or carcinoma[1]	0.074	NE
Lung adenoma	0.070	0.037
Lung adenoma or carcinoma[1]	0.070	0.048
All benzofuran-related tumor sites	**0.63**	---

Bolding indicates value selected as the basis of the NSRL.

[1] Distributions of q_1 combined using Monte Carlo analysis that were used in deriving the potency for "all benzofuran-related tumor sites."

[2] NE, not estimated.

A cancer potency estimate of 0.63 $(mg/kg\text{-}day)^{-1}$ for male mice was derived from the combined distribution of cancer potency estimates for all benzofuran-related tumor sites (NTP, 1989) (Figure 1).

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The combined cancer potency estimate for all benzofuran-related tumor sites, 0.63 $(mg/kg\text{-}day)^{-1}$, derived above was used to calculate the NSRL for benzofuran (1.1 µg/day).

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR BENZOFURAN

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 benzofuran are outlined in this Appendix.

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

NTP (1989) provided estimates of the average daily dose administered to the mice. Male $B6C3F_1$ mice received 0, 60 or 120 mg/kg body weight in corn oil, five days per week for two years. Female $B6C3F_1$ mice received 0, 120 or 240 mg/kg body weight in corn oil, five days per week for two years. The daily intake estimates were adjusted to lifetime daily intake estimates by multiplying the administered doses by (5/7) to adjust the five-day-per-week dosing regimen to

an equivalent cumulative dose that would be expected from continuous dosing (i.e., seven days per week).

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 $(mg/kg\text{-}day)^{-1}$:

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

Average body weights for male mice, 0.038 kg, and female mice, 0.030 kg, were estimated from weekly body weight data provided in the NTP Technical Report (Table 13 of NTP, 1989). A default body weight of 70 kg for humans was assumed (Gold and Zeiger, 1997).

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:

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

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