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

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Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency

SUMMARY OF FINDINGS

The cancer potency of bromoform was estimated from dose-response data for gastrointestinal tumors among female rats exposed orally to bromoform by gavage (NTP, 1989). 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. 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 the corresponding NSRL are given in Table 1.

Table 1. Cancer Potency and NSRL for Bromoform.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
Bromoform	0.011	64

INTRODUCTION

This report describes the derivation of a cancer potency estimate and NSRL for bromoform (CAS No. 75-25-2; molecular weight 252.7, synonyms: tribromomethane, methyl tribromide). "Bromoform" was listed on April 1, 1991, as a chemical known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). Bromoform has been used mainly as a chemical intermediate and a solvent and is a drinking water contaminant as a disinfection byproduct (NTP, 1989).

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

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

No epidemiological studies which have directly examined the carcinogenicity of bromoform have been identified in the scientific literature. Several studies have examined the relationship between the consumption of tap water containing trihalomethanes (chloroform and three

brominated trihalomethanes, including bromoform) and cancer incidence, although each is confounded by the presence of multiple cancer causing compounds (reviewed by Mills *et al.*, 1998). Tumors of the bladder, colon, and rectum have been associated with human exposure to chlorinated drinking water (reviewed by Boorman *et al.*, 1999).

The most suitable carcinogenicity data for the assessment of cancer risk to humans from exposure to bromoform come from the studies conducted in rats by the National Toxicology Program (NTP, 1989) showing significant increases in adenomatous polyps of the large intestine in female rats and adenomatous polyps and carcinomas of the large intestine in male rats. NTP also conducted simultaneous studies in male and female $B6C3F_1$ mice in which no significant increases in tumor incidence were observed. The adequacy of the dosing for the male mice in the NTP studies has been called into question since few signs of toxicity were observed. A positive study demonstrating carcinogenic potential was also reported by Theiss *et al.* (1977) using Strain A mice, a strain highly sensitive to certain carcinogens, in which a treatment-related increase in lung adenomas was observed following intraperitoneal injection of bromoform. The high sensitivity of this strain makes it less suitable for dose-response assessment.

Briefly, in the NTP rat studies, bromoform was administered by oral gavage in corn oil to male and female F/344 rats (50 animals/sex) at dose levels of 100 and 200 mg bromoform/kg bodyweight five days per week for 103 weeks. A vehicle control group (50 animals/sex) was included in the studies. Survival was significantly reduced among males in the high dose group relative to the control animals after week 91 of the study. No other group showed significantly reduced survival. Tumors of the large intestine developed in both male and female rats treated with bromoform (see Table 2). Among female rats, a significant increase in adenomatous polyps of the large intestine was observed in the high dose group relative to control animals. Two adenocarcinomas of the large intestine were also observed in the high dose group, although the increase was not statistically significant. The combined incidence of these two types of tumors of the large intestine was significantly increased. Both the combined incidences and the incidences of adenomatous polyp showed a significantly increasing trend with dose by an exact test for linear trend. In male rats, the incidence of intestinal adenomas and carcinomas was increased at the high dose, but the increase was not statistically significant and there was a dose-related increase incidence by an exact test for linear trend. Neoplastic nodules of the liver also occurred in four low-lose and two high-dose female rats compared to none in the control group, although this increase was not statistically significant.

Table 2. Tumors of the Large Intestine in Rats Treated by Gavage with Bromoform for Two Years (NTP, 1989).

Sex/Species/Tumor Type	Administered Dose (mg/kg)[a]			Trend[b]
	Controls	100	200	
Male Rats				
Adenomatous polyp	0/43	0/49	2/39	N.S.
Adenocarcinoma	0/34	0/30	1/12	N.S.
Polyp or adenocarcinoma	0/43	0/49	3/39	p = 0.025
Female Rats				
Adenomatous polyp	0/45	1/34	6/41[c]	p = 0.0029
Adenocarcinoma	0/34	0/27	2/28	N.S.
Polyp or adenocarcinoma	0/45	1/34	8/41[c]	p = 0.00034

[a] Incidence reported here is the number of tumor-bearing animals per total number of animals with tissues examined in that group considered "at risk" (surviving to the time of first tumor development; 76 weeks for adenomatous polyps and 104 weeks for adenocarcinoma of the large intestine in male rats; and 91 weeks for adenomatous polyps and 106 weeks for adenocarcinoma of the large intestine in female rats).

[b] p-Value of exact test for linear trend; N.S. = not significant ($p > 0.05$).

[c] Statistically significant increase above controls by Fisher's exact test ($p < 0.05$).

Tumors of the large intestine are rare in F344/N rats. NTP (1989) reports that the historical incidences of intestinal tumors in corn oil gavage studies at the laboratory that performed the bromoform studies were 0/285 for male rats and 0/282 for female rats. Overall historical control incidence (as of the time of the NTP studies) was 3/1873 (0.2%) for males and 0/1888 for females.

APPROACH TO DOSE-RESPONSE ANALYSIS

Numerous lines of evidence strongly suggest that bromoform is a mutagenic and genotoxic compound.

In Vitro Assays in Bacteria

Positive evidence of mutagenicity from reverse mutation assays with *Salmonella typhimurium* has been demonstrated in strain TA100 without metabolic activation (Simmon *et al.*, 1978; Simmon and Tardiff, 1978; Rapson *et al.*, 1980; Ishidate *et al.*, 1982; Haworth *et al.*, 1983; Le Curieux *et al.*, 1995; Varma *et al.*, 1988). Other *Salmonella* strains, such as TA98, TA1535, and TA1537 have not consistently tested positive in these assays. Bromoform is a volatile compound, so an important aspect to the mutagenicity testing is the use of a closed system that minimizes loss of the test compound. Many of the reported *in vitro* studies of bromoform mutagenicity do not state whether a closed system was used.

In Vitro Assays in Mammalian Cells

There is mixed evidence for increases in sister chromatid exchange from treatment *in vitro* with bromoform, with some assays showing an increase (human lymphocytes: Morimoto and

Koizumi, 1983) and others reporting equivocal or negative results (Chinese hamster ovary cells: NTP, 1989; human lymphocytes: Landi *et al.*, 1999a; Landi *et al.*, 1999b). Increases in chromosomal aberrations have been observed following treatment of Chinese hamster fibroblasts with bromoform (Ishidate *et al.*, 1982), but not Chinese hamster ovary cells (Galloway *et al.*, 1985; NTP, 1989).

***In Vivo* Tests for Genotoxicity**

Bromoform treatment increased chromosomal aberrations in bone marrow cells of rats treated by intraperitoneal injection and by oral administration (Fujie *et al.*, 1990). Sister chromatid exchanges and micronuclei were increased in bone marrow cells from mice treated with bromoform (Morimoto and Koizumi, 1983; NTP, 1989). The induction of micronuclei in mouse bone marrow cells has not been consistently observed, however (Hayashi *et al.*, 1988; Stocker *et al.*, 1997).

Herren-Freund and Pereira tested bromoform for "initiation" activity in a two-stage rat model in which two-thirds partial hepatectomy (and subsequent regeneration) followed eight days later by treatment with 500 ppm phenobarbital in the drinking water served as the "promotion" stimulus (Herren-Freund and Pereira, 1986). Induction of γ-glutamyl transpeptidase (GGT)-positive foci in the liver was measured. The experiment involved treatment of nine rats with a single dose of 1.0 mmol bromoform (~250 mg) per kilogram bodyweight (route not stated) 24 hours following partial hepatectomy. No significant increase in GGT-positive foci was observed in the livers of bromoform treated animals.

No damage to DNA was reported in the renal cells of rats exposed to bromoform (Potter *et al.*, 1996) and unscheduled DNA synthesis was not induced in the hepatocytes of rats exposed to bromoform (Stocker *et al.*, 1997).

Investigations have attempted to identify metabolic paths that may be important in mediating the mutagenicity of bromoform. An important study published by DeMarini *et al.* (1997) examined a possible role for glutathione *S*-transferase-theta (GSTT1-1) in the mutagenicity of bromoform in *Salmonella.* Mutant strains were constructed bearing functional or non-functional copies of the rat glutathione *S*-transferase-theta gene (*GSTT1-1*). *Salmonella* bearing the functional *GSTT1-1* gene produced nearly a hundred-fold greater number of revertant colonies following treatment with bromoform than the control bacteria. These mutations were found to be largely GC→AT transitions, compared to mutations induced by dichloromethane, which consist of a relatively small proportion of GC→AT transitions. These results suggest that GSTT1-1 may play a large role in mediating the mutagenicity of bromoform and that GSTT1-1-mediated metabolism of bromoform results in the formation of a specific type of mutational lesion in *Salmonella.*

It is unknown whether GSTT1-1 serves to activate bromoform in humans. Humans are known to bear deletion polymorphisms of *GSTT1-1*, with 10 to 60 percent of the population homozygous for the deletion, depending on ethnicity (Landi, 2000). If GSTT1-1 activity were important in bioactivation to DNA damaging compounds, individuals homozygous for the deletion could theoretically be at lower cancer risk than those without the deletion. Heterozygous and homozygous individuals without the deletion express intermediate and higher levels of the enzyme, respectively.

The overall evidence regarding the potential for bromoform to cause genotoxicity favors its characterization as a chemical capable of causing DNA damage. Beyond this, it is unknown how bromoform causes the intestinal tumors that have been observed in rats treated with the chemical in lifetime bioassays, so the information on the precise mechanism of carcinogenicity is insufficient 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. For these reasons, the linearized multistage model (*i.e.*, the default model) has been applied to the tumor response data to estimate the carcinogenic potency of this compound. The selection of the tumor site and the sensitive sex and species is clear from the available data, tumors of the large intestine in female rats, so the model has been fit to these data for the potency estimate.

DOSE RESPONSE ASSESSMENT

Since female rats were the more sensitive species to the carcinogenic effect of bromoform, the tumors that developed in these animals were used as the basis for the potency estimate. Using the incidence data for combined tumors of the large intestine (Table 2) and the lifetime average dose estimates of 0, 71.4, and 142.8 mg/kg-day (see Appendix for calculation), a q_1^* of 0.0016 $(mg/kg\text{-}day)^{-1}$ was calculated. Using the interspecies conversion described in the Appendix, a human cancer potency of 0.011 $(mg/kg\text{-}day)^{-1}$ was calculated.

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 of 0.011 $(mg/kg\text{-}day)^{-1}$ based on adenomatous polyps and adenocarcinomas of the large intestine in female rats was used to calculate the NSRL for bromoform (64 μg/day).

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR BROMOFORM

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 bromoform are 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, 1987; 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

In the 1989 NTP studies which form the basis for the estimation of the cancer potency, bromoform was administered to female F/344 rats by oral gavage in a corn oil vehicle for five days per week for 103 weeks. The administered doses of 0, 100, and 200 mg/kg body weight correspond to lifetime average doses of 0, 71.4 and 143 mg/kg-day, respectively, based upon the fraction of time the animals were dosed (5 of 7 days).

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

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

An average body weight for female rats of 0.25 kg was estimated from weekly body weight data provided in the NTP Technical Report (Table 11 of NTP, 1989). A default body weight of 70 kg for humans was assumed (Gold and Zeiger, 1997).

A.3 Risk-Specific Intake Level Calculation

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

$$I = \frac{R \times bw_h}{q_{human}} \quad (5)$$

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:

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

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