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

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SUMMARY OF FINDINGS

The Office of Environmental Health Hazard Assessment (OEHHA) estimated the human cancer potency of bromoethane and used it to calculate a "No Significant Risk Level" (NSRL) for the chemical. To estimate the human cancer potency, OEHHA applied the linearized multistage model to dose-response data for uterine tumors (adenomas, adenocarcinomas or squamous cell carcinomas, combined) in female $B6C3F_1$ mice exposed to bromoethane via inhalation. The potency derivation takes into account differences in body size between humans and experimental animals.

The Proposition 65 NSRL is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer (the level of exposure estimated to cause one additional cancer case in a population of 100,000 people exposed daily over a lifetime). The human cancer potency estimate for bromoethane is 0.0073 milligrams per kilogram per day $(mg/kg\text{-}day)^{-1}$ and the corresponding NSRL is 96 micrograms per day (µg/day).

Table 1. Human cancer potency and NSRL for bromoethane.

Chemical	Cancer potency $(mg/kg\text{-}day)^{-1}$	NSRL (µg/day)
Bromoethane	0.0073	96

INTRODUCTION

This report describes the derivation of a cancer potency estimate and NSRL for bromoethane (CAS number 74-96-4, molecular weight 109.0). Bromoethane, also known as ethyl bromide, was listed on December 22, 2000 as known to the State to

cause cancer under Proposition 65 (formally known as the Safe Drinking Water and Toxic Enforcement Act of 1986; California Health and Safety Code 25249.5 *et seq*.). Bromoethane is mainly used as an ethylating agent in the production of organic chemicals. Bromoethane is also naturally emitted from some marine algae (IARC, 1991). The U.S. production/import volume was reported to be between 1 and 10 million pounds in 2006 (U.S. EPA, 2006).

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

There are no human carcinogenicity studies of bromoethane. The only animal bioassay studies available are the inhalation studies by the National Toxicology Program (NTP, 1989) in which bromoethane was administered to male and female F344/N rats and $B6C3F_1$ mice.

Groups of 50 male and 50 female rats and mice were exposed via inhalation to bromoethane six hours per day, five days per week at 0, 100, 200 or 400 parts per million (ppm) for 104 (rats) or 103 (mice) weeks (NTP, 1989). NTP found clear evidence of carcinogenic activity in female mice based on a statistically significant increase in rare uterine tumors (adenomas, adenocarcinomas or squamous cell carcinomas combined).

The dose-response data for the study in female $B6C3F_1$ mice are presented in Table 2. Some of the adenocarcinomas metastasized to other organs including the lung. The survival of female mice dosed at 400 ppm was lower than the control and other dose groups beginning around week 90, with a highly significant difference by the end of the study. NTP concluded that the higher mortality of the 400 ppm group was likely related to the uterine cancer.

Table 2. Incidence of uterine tumors in female B6C3F$_1$ mice treated with bromoethane via inhalation for 103 weeks (NTP, 1989).

Sex, strain, species	Tumor site and type	Administered dose (ppm)	Average daily dose[a] (mg/kg-day)	Tumor incidence[b]	Statistical significance[c]
Female B6C3F$_1$ mice	Uterine adenomas, adenocarcinomas or squamous cell carcinomas (combined)	0	0	0/49	$p < 0.001$[d]
		100	101	4/45	$p = 0.05$
		200	201	5/44	$p = 0.02$
		400	403	27/45	$p < 0.001$

[a] Bromoethane was administered to female mice six hours per day, five days per week, for 103 weeks; animals were sacrificed at 104 weeks. Average daily dose was calculated as described in the Appendix.

[b] The denominator represents the number of female mice alive at the time of the appearance of the first uterine tumor (week 82).

[c] Results of pairwise comparison using Fisher's Exact Test, except as noted.

[d] Exact trend test p-value.

APPROACH TO DOSE-RESPONSE ANALYSIS

This section reviews the genotoxicity data on bromoethane and other data relevant to possible mechanisms of carcinogenicity for the purpose of determining the most appropriate approach for dose-response analysis.

NTP (1989) and the International Agency for Research on Cancer (IARC, 1991) summarized bromoethane genotoxicity tests available at that time. Bromoethane was found to be mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 with and without metabolic activation, but not in strains TA98 or TA1537 (NTP, 1989; IARC, 1991). Bromoethane also tested negative in the sex-linked recessive lethal mutation assay in *Drosophila melanogaster* (IARC, 1991). However, bromoethane induced sister chromatid exchanges in Chinese Hamster Ovary (CHO) cells (with and without metabolic activation), but not chromosomal aberrations (NTP, 1989). More recent studies found that bromoethane did not induce micronuclei in CHO cells (Sobol *et al.,* 2007) but did cause damage to calf thymus DNA (Kailasam and Rogers, 2007) and also induced DNA deletions in *Saccharomyces cerevisiae* (Sobol *et al.,* 2007). These findings together suggest that a genotoxic mechanism of action for the carcinogenicity of bromoethane is plausible.

Bucher *et al.* (1995) examined the possibility that bromoethane caused uterine carcinogenesis by an early change in circulating sex hormones, but found "no convincing evidence" that tumorigenic doses of bromoethane (or chloroethane) caused such changes. More recently, Aoyama *et al.* (2005) concluded that bromoethane

uterine carcinogenesis may be related to upregulation of the alpha estrogen receptor (ERα) in the uterus.

There is insufficient information on the precise mechanism(s) of bromoethane-induced 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, a linearized multistage model and interspecies scaling has been applied (see Appendix).

DOSE-RESPONSE ASSESSMENT

Animal and human cancer potency estimates were derived for bromoethane by fitting the multistage model to the dose-response data from the NTP (1989) study in female mice (Table 2). Multiplying the animal cancer potency estimate derived from the study in female mice by the applicable interspecies scaling factor (see Appendix) gives an estimate of human cancer potency.

The results are summarized in Table 3 below. More details on the derivation of the estimates are provided in the Appendix.

Table 3: Animal and human cancer potency estimates for bromoethane.

Sex, strain, species	Tumor site and type	Animal cancer potency $(mg/kg\text{-}d)^{-1}$	Human cancer potency $(mg/kg\text{-}d)^{-1}$
Female $B6C3F_1$ mice	Uterine adenomas, adenocarcinomas, or squamous cell carcinomas (combined)	0.00106	0.0073

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The human cancer potency estimate of 0.0073 $(mg/kg\text{-}d)^{-1}$ for bromoethane, based on data from female mice, was used to calculate the NSRL for this chemical. The value of 96 µg/day was derived as shown below.

$$\text{NSRL} = \frac{10^{-5} \times 70\,\text{kg}}{0.0073\,(\text{mg/kg-day})^{-1}} \times 1000\,\mu\text{g/mg} = 96\,\mu\text{g/day}$$

REFERENCES

Aoyama H, Couse JF, Hewitt SC, Haseman JK, He H, Zheng X, Majstoravich S, Korach KS, Dixon D (2005). Upregulation of estrogen receptor expression in the uterus of ovariectomized B6C3F_1 mice and Ishikawa cells treated with bromoethane. *Toxicology and Applied Pharmacology* **209**:226-235.

Bucher JF, Morgan DL, Adkins B, Travlos GS, Davis BJ, Morris R, Elwell MR (1995). Early changes in sex hormones are not evident in mice exposed to the uterine carcinogens chloroethane or bromoethane. *Toxicology and Applied Pharmacology* **130**:169-173.

International Agency for Research on Cancer (IARC, 1991). Bromoethane. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Chlorinated Drinking Water; Chlorination By-products; Some Other Halogenated Compounds; Cobalt and Cobalt Compounds.* Volume 52. IARC, Lyon, France, pp. 299.

Kailasam S, Rogers KR (2007). A fluorescence-based screening assay for DNA damage induced by genotoxic industrial chemicals. *Chemosphere* **66**:165-171.

National Toxicology Program (NTP, 1989). *Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS NO. 74-96-4) in F344 Rats and B6C3F1 Mice (Inhalation Studies).* Technical Report Series No. 363. NIH Publication No. 90-2818. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health.

Sobol Z, Engel ME, Rubiski E, Ku WW, Aubrecht J, Schiestl RH (2007). Genotoxicity profiles of common alkyl halides and esters with alkylating activity. *Mutation Research* **633**(2): 80-94.

U.S. Environmental Protection Agency (U.S. EPA, 2006). Non-Confidential 2006 Inventory Update Reporting Data. National Chemical Information. Available at: http://cfpub.epa.gov/iursearch/index.cfm.

APPENDIX: METHODOLOGY USED TO DERIVE THE NSRL FOR BROMOETHANE

Procedures for the development of Proposition 65 NSRLs are described in regulation in Title 27, California Code of Regulations (CCR), Sections 25701 and 25703. Consistent with these procedures, the specific methods used to derive the NSRL for bromoethane 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 (California Department of Health Services [CDHS], 1985; Anderson *et al.*, 1983):

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

with constraints, $q_i \geq 0$ for all i. The q_i are parameters of the model, which are taken to be constants and are estimated from the animal cancer bioassay data. With four dose groups, as is the case with the NTP (1989) studies of bromoethane, the linearized multistage model defaults to three stages, or four parameters, q_0, q_1, q_2, and q_3. The parameter q_0 provides the basis for estimating the background lifetime probability of the tumor (i.e., $1 - \exp[-(q_0)]$). The parameter q_1 is, for small doses, the ratio of excess lifetime cancer risk to the average daily dose received. The upper 95% confidence bound on q_1, estimated by maximum likelihood techniques, is referred to here as q_{animal}. When dose is expressed in units of mg/kg-day, the parameters q_1 and q_{animal} are given in units of $(\text{mg/kg-day})^{-1}$. Details of the estimation procedure are given in Crump (1984) and Crump *et al.* (1977). To estimate risk at low doses, potency is multiplied by average daily dose.

Calculation of average daily dose

The lifetime average dose in units of mg/kg-day of bromoethane was calculated for each of the relevant dose groups, based on the dose level, duration and regimen described in the NTP (1989) studies. For bromoethane, the average body weight for female mice of 0.0315 kg was calculated based on data for control female mice provided in NTP (1989).

The inhalation rate (IR), in m^3/day, for female mice was calculated based on the equation of Anderson *et al*. (1983), which was derived using experimental data on animal breathing rates (mg/m^3) and corresponding body weights (kg):

$$IR_{mice} = 0.0345 \times (bw_{mice}/0.025)^{2/3}$$

The constant 0.0345 is in m^3/day and the constant 0.025 is in kg. The calculated inhalation rate for female mice was 0.0402 m^3/day. Lifetime average doses (D_{avg}) were determined by multiplying the chamber air concentration (C_{air}) of bromoethane in units of ppm by the following factors: bromoethane conversion factor of 4.46 (mg/m^3)/ppm (NIOSH, 2005); the inhalation rate for female mice divided by the body weight; 6/24 to account for the six hour per day exposure; 5/7 to account for a five day per week dosing; and 103/104 to account for the exposure period of 103 weeks out of a 104 week study length. The lifetime average dose (mg/kg/day) calculation for female mice is:

$$D_{avg} = C_{air}\,(ppm) \times 4.46 \frac{(mg/m^3)}{ppm} \times \frac{IR_{mice}}{bw_{mice}} \frac{(m^3/day)}{kg} \times \frac{6}{24} \times \frac{5}{7} \times \frac{103}{104}$$

A.2 Interspecies Scaling

Once a potency value is estimated in animals following the techniques described above, the 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-fourth power when animal potency is expressed in units $(mg/kg\text{-}day)^{-1}$ (Title 27 CCR Section 25703(a)(6)):

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

An example calculation using the female mouse animal cancer potency of $0.00106\ (mg/kg\text{-}day)^{-1}$, corresponding body weight of 0.0315 kg and the default human body weight of 70 kg is shown below:

$$q_{human} = 0.00106\ (mg/kg\text{-}day)^{-1} \bullet (70\ kg\ /0.0315\ kg)^{1/4} = 0.0073\ (mg/kg\text{-}day)^{-1}$$

A.3 Risk-Specific Intake Level Calculation

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

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

where bw_h is the human body weight (in kg), and q_{human} (in units $[mg/kg\text{-}day]^{-1}$) is the cancer potency estimate for humans.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the NSRL for cancer under Proposition 65 (Section 25703). Thus for a 70 kg person, the NSRL (in μg/day) is given by:

$$NSRL = \frac{10^{-5} \times 70\,kg}{q_{human}} \times 1000\,\mu g/mg$$

Applying this formula and converting to micrograms per day results in an NSRL of 96 μg/day for bromoethane.

APPENDIX REFERENCES

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group (1983). Quantitative approaches in use to assess cancer risk. *Risk Analysis* **3**:277-295.

California Department of Health Services (CDHS, 1985). Guidelines for Chemical Carcinogen Risk Assessment and Their Scientific Rationale. California Department of Health Services, Health and Welfare Agency, Sacramento, CA.

Crump KS (1984). An improved procedure for low-dose carcinogenic risk assessment from animal data. *J Environ Pathol Toxicol Oncol* **5**:339-48.

Crump KS, Guess HA, Deal LL (1977). Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. *Biometrics* 33:437-451.

National Institute for Occupational Safety and Health (NIOSH, 2005). NIOSH Pocket Guide to Chemical Hazards. Bromoethane. Publication No. 2005-149. Department of Health and Human Services, Centers for Disease Control & Prevention. Available at: http://www.cdc.gov/niosh/npg/npgd0265.html. Accessed June, 2012.

National Toxicology Program (NTP, 1989). *Toxicology and Carcinogenesis Studies of Bromoethane (Ethyl Bromide) (CAS NO. 74-96-4) in F344 Rats and B6C3F1 Mice (Inhalation Studies).* Technical Report Series No. 363. NIH Publication No. 90-2818. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health.