NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN 2-METHYLAZIRIDINE (PROPYLENEIMINE)

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SUMMARY OF FINDINGS

The cancer potency of 2-methylaziridine (propyleneimine) was estimated from the dose-response data of Weisburger *et al*. (1981). 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 experimental 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 corresponding NSRL are given in Table 1.

Table 1. Human Cancer Potency and NSRL for 2-Methylaziridine.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (µg/day)
2-Methylaziridine	25	0.028

INTRODUCTION

This report describes the derivation of a cancer potency estimate and NSRL for 2-methylaziridine (CAS number 75-55-8, molecular weight 57.1). "2-Methylaziridine" was listed on January 1, 1988 as a chemical known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). A frequently used synonym for 2-methyl-aziridine is propyleneimine. 2-Methylaziridine has been used as a chemical intermediate for the production of polymers, coatings, adhesives, and textiles (IARC, 1999; HSDB, 2002).

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 is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

The results of carcinogenicity studies of 2-methylaziridine in male and female rats were published in a preliminary report by Ulland *et al*. (1971) and in a final report by Weisburger *et al*. (1981). No other studies were available for estimating human cancer potency for the purposes of Proposition 65.

Groups of 26 male and 26 female Charles River CD rats were exposed to 2-methylaziridine in water twice weekly via gavage. Control animals (16 males and 16 females) were maintained in the same room as the treated groups. The low-dose groups received 10 mg/kg twice weekly for the first eight weeks of the exposure period. The dose was then increased to 12.5 mg/kg twice weekly for 12 weeks, followed by a two-week period of non-exposure. Animals were then treated with 10 mg/kg twice weekly for 36 weeks. The total exposure period, including the two-week period of no dosing, was 58 weeks for the low-dose groups. Animals in the high-dose groups were administered 20 mg/kg twice weekly for the first eight weeks of the exposure period. The dose was then increased to 25 mg/kg for 12 weeks, followed by a two-week period of non-exposure. Animals were then treated with 20 mg/kg for five weeks. The total exposure period, including the two-week period of no dosing, was 27 weeks for the high-dose groups. The shortened exposure period in the high-dose groups was due to the poor condition of the animals and the observation of palpable masses (Ulland *et al.*, 1971). Surviving animals in all groups were killed at week 60.

Treatment with 2-methylaziridine significantly increased the mortality of the animals. These studies were terminated at 60 weeks, because only a few animals were still alive at that time (Weisburger *et al.*, 1981). Survival in the low dose-groups was 42 and 12 percent for males and females, respectively, at week 52. Survival in the high-dose groups at 52 weeks was 12 and eight percent for males and females, respectively. Survival at 52 weeks among negative controls for all studies being conducted concurrently was reported as 97% and 99% percent for males and females, respectively.

Significant increases were observed in the incidences of mammary adenocarcinoma in treated female rats and leukemia in treated male rats. The authors considered increases in the following tumors to be probably related to the administration of 2-methylaziridine, although the increases were not statistically significant: malignant gliomas of the cerebrum, squamous cell carcinomas of the ear duct, and adenocarcinomas of the small intestine. Females appeared to be more sensitive than males to the carcinogenic effects of 2-methylaziridine. The dose-response data for mammary adenocarcinoma in female rats and leukemia in male rats are reported in Tables 2 and 3.

Table 2. Mammary Adenocarcinoma in Female Charles River CD Rats Treated by Gavage with 2-Methylaziridine (Weisburger *et al.*, 1981).

Administered Dose[1] (mg/kg)	Lifetime Average Daily Dose[2] (mg/kg-day)	Incidence of Mammary Adenocarcinoma	Statistical Significance[4]
0	0	0/16	---
10	2.81	21/26	$p < 0.001$
20	2.67[3]	10/26	$p < 0.01$

1 The administered dose is the dose of 2-methylaziridine given by gavage at the start of the experiment. The doses were changed in each group during the experiment.

2 The average daily dose was calculated as the time-weighted average dose over the 60-week experiment based on data provided in Weisburger *et al.* (1981). Details on the calculation are given in the Appendix.

3 Due to the condition of the animals in the high dose group, treatment was stopped after week 27 of the study. Twenty-four of 26 high-dose female rats had died by week 52; the two remaining animals were killed at week 60. Averaging the administered dose over the length of the study results in an underestimate of the average dose received by most of the animals in this group.

4 p-value listed next to dose groups is the result of pairwise comparison with the control group using the Fisher exact test.

Table 3. Leukemia in Male Charles River CD Rats Treated by Gavage with 2-Methylaziridine (Weisburger *et al.*, 1981).

Administered Dose[1] (mg/kg)	Lifetime Average Daily Dose[2] (mg/kg-day)	Incidence of Leukemia	Statistical Significance[4]
0	0	0/16	---
10	2.81	4/26	$p = 0.13$
20	2.67[3]	6/26	$p < 0.05$

1 The administered dose is the dose of 2-methylaziridine given by gavage at the start of the experiment. The doses were changed in each group during the experiment.

2 The average daily dose was calculated as the time-weighted average dose over the 60-week experiment based on data provided in Weisburger *et al.* (1981). Details on the calculation are given in the Appendix.

3 Due to the condition of the animals in the high dose group, treatment was stopped after week 27 of the study. Twenty-three of 26 high-dose male rats studied had died by week 52; the three remaining animals were killed at week 60. Averaging the administered dose over the length of the study results in an underestimate of the average dose received by most of the animals in this group.

4 p-value listed next to dose groups is the result of pairwise comparison with the control group using the Fisher exact test.

APPROACH TO DOSE-RESPONSE ANALYSIS

2-Methylaziridine induced unscheduled DNA synthesis in human fibroblasts *in vitro* (CCRIS, 1994) and transformations in mouse CH3/10T1/2 cells under replating conditions (IARC, 1999) and in mouse BALB/c-3T3, Syrian hamster embryo cells, and rat embryo cells (GENE-TOX, 1995). 2-Methylaziridine induced sex linked recessive lethal mutations in a DNA repair-deficient strain of *Drosophila melanogaster* following inhalation, and somatic mutations in other strains following ingestion (IARC, 1999). 2-Methylaziridine was genotoxic towards *Saccharomyces cerevisiae*, and mutagenic towards *Salmonella typhimurium* and *Escherichia coli* under standard *in vitro* conditions (IARC, 1999). It also induced mutations in *Saccharomyces cerevisiae* and *Salmonella typhimurium* in mouse host-mediated assays (IARC, 1999).

No information on the metabolism of 2-methylaziridine was located. The compound is a cyclic secondary amine and may be susceptible to oxygenation by flavin-containing monooxygenase (Ziegler, 1980). Rapid percutaneous penetration has been reported (HSDB, 2002).

The above 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 for 2-methylaziridine based on the data for mammary adenocarcinoma in female rats and leukemia in male rats (Weisburger *et al.*, 1981). Survival was compromised in all treated groups, but individual animal data are not available for analysis. In the high-dose groups, the condition of the animals was so poor that the exposure was terminated early. Ulland *et al*. (1971) postulated that the lower incidence of mammary adenocarcinoma observed in high-dose females compared to low-dose females may have been due to the shortened exposure period in the high-dose group. Weisburger *et al.* (1981) commented that the inversion in the dose-response for mammary adenocarcinoma was likely due to the high early mortality in high-dose females. There were also problems in obtaining a reasonable estimate of lifetime average dose in the high-dose groups, because significant numbers of animals died early. Averaging the exposure over 60 weeks would underestimate dose for these animals. Because of the uncertainty in the interpretation of the dose-response data in the high-dose groups, they were dropped from the analyses of both the male and female rat data.

Potency estimates are summarized in Table 4. The human cancer potency is estimated to be 25 $(mg/kg\text{-}day)^{-1}$ based on the data for female rats, the more sensitive sex. Because of the survival problems in the Weisburger *et al.* (1981) study of female rats, this cancer potency value is likely to be an underestimate.

Table 4. Human Cancer Potency Estimates for 2-Methylaziridine Based on Weisburger *et al*. (1981).

Sex, Species	Tumor	Cancer Potency Estimate $(mg/kg\text{-}day)^{-1}$
Female rat	Mammary adenocarcinoma	**25**
Male rat	Leukemia	3.0

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 25 $(mg/kg\text{-}day)^{-1}$ based on mammary adenocarcinoma in female rats was used to calculate the NSRL of 0.028 µg/day for 2-methylaziridine.

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR 2-METHYLAZIRIDINE

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 2-methylaziridine 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

Weisburger *et al.* (1981) changed the doses of 2-methylaziridine several times during treatment for both the low- and high-dose groups of rats. The weighted mean doses per gavage treatment

were reported to be 10.17 mg/kg for the low-dose groups over a 58-week exposure period and 20.74 mg/kg over a 27-week exposure period for the high-dose groups. The experiment was terminated at 60 weeks. The calculations of the weighted mean doses and the lifetime average doses are shown below:

Low- dose group:

$$\frac{(10\ \text{mg/kg} \times 8\ \text{wks} + 12.5\ \text{mg/kg} \times 12\ \text{wks} + 0\ \text{mg/kg} \times 2\ \text{wks} + 10\ \text{mg/kg} \times 36\ \text{wks})}{58\ \text{wks}} = 10.17\ \text{mg/kg}$$

$$10.17\ \text{mg/kg - dose} \times \frac{2\ \text{doses}}{7\ \text{days}} \times \frac{58\ \text{weeks}}{60\ \text{weeks}} = 2.81\ \text{mg/kg - day}$$

High-dose group:

$$\frac{(20\ \text{mg/kg} \times 8\ \text{wks} + 25\ \text{mg/kg} \times 12\ \text{wks} + 0\ \text{mg/kg} \times 2\ \text{wks} + 20\ \text{mg/kg} \times 5\ \text{wks})}{27\ \text{wks}} = 20.74\ \text{mg/kg}$$

$$20.74\ \text{mg/kg - dose} \times \frac{2\ \text{doses}}{7\ \text{days}} \times \frac{27\ \text{weeks}}{60\ \text{weeks}} = 2.67\ \text{mg/kg - day}$$

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

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

Human body weight is assumed to be 70 kg (Gold and Zeiger, 1997). Weisburger *et al.* (1981) reported mean body weights for control Charles River CD rats at 51 weeks to be 0.402 kg for females and 0.709 kg for males.

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 human 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:

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

APPENDIX REFERENCES

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