NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN 4-METHYLIMIDAZOLE

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

The human cancer potency of 4-methylimidazole was estimated and used to calculate a "No Significant Risk Level" (NSRL). The human cancer potency was estimated from dose-response data using the linearized multistage model for combined alveolar/bronchiolar adenomas or carcinomas in male $B6C3F_1$ mice exposed via their feed (National Toxicology Program [NTP], 2007). 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 NSRL for 4-methylimidazole is calculated to be 16 µg/day.

Table 1. Cancer potency and NSRL for 4-methylimidazole.

Chemical	Cancer Potency (mg/kg-day)$^{-1}$	NSRL (μg/day)
4-Methylimidazole	0.045	16

INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for 4-methylimidazole (CAS No. 822-36-6). 4-Methylimidazole was listed on January 7, 2011 as a chemical known to the state of California 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.*).

4-Methylimidazole is used in the manufacture of pharmaceuticals, photographic chemicals, dyes and pigments, cleaning and agricultural chemicals, and rubber. Additionally, it is a fermentation by–product in ammoniated livestock feed and in certain food products including caramel coloring, soy sauce, Worcestershire sauce, wine, and ammoniated molasses (NTP, 2007).

The studies available for cancer dose-response assessment and the derivations of the cancer potency estimate and NSRL are discussed below. A detailed description of the methodology used is provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

There are no human carcinogenicity studies of 4-methylimidazole. The only cancer bioassays available for estimating the potency of 4-methylimidazole were conducted by NTP (2007) and are discussed in detail below. These carcinogenicity data were determined to be suitable for human cancer potency assessment.

NTP (2007) exposed male and female F344/N rats and B6C3F$_1$ mice (50 animals/group/sex) to 4-methylimidazole via their diet for 106 weeks. As reported by NTP, doses administered to male rats were 0, 30, 55, and 115 mg/kg-day and doses administered to female rats were 0, 60, 120, and 260 mg/kg-day. Also as reported by NTP, doses administered to male and female mice were 0, 40, 80, and 170 mg/kg-day. Feed was available *ad libitum* and feed consumption was similar overall in dosed groups and control groups for both rats and mice, respectively, except for the high-dose group of female rats, in which feed consumption was observed to be less than the controls.

Survival of dosed groups was similar to that of the corresponding control groups in both rats and mice. Mean body weights for male rats in the low- and mid-dose groups and female rats in the mid- and high-dose groups were less than those of controls for the duration of the study and mean body weights of female rats in the low-dose group were less than those of controls after week 41. Mean body weights of male and female mice in the high-dose groups were less than those of controls after 17 weeks and mean body weights of female mice in the low- and mid-dose groups were less than those of controls after weeks 85 and 65, respectively.

NTP (2007) concluded that there is clear evidence of the carcinogenic activity of 4-methylimidazole in male and female B6C3F$_1$ mice. In male mice, the incidence of combined alveolar/bronchiolar adenoma or carcinoma was significantly increased in the high-dose group [$p < 0.01$] and occurred with a positive trend [$p < 0.01$]. Tumor incidences in treated males exceeded the NTP historical control incidence in male B6C3F$_1$ mice for combined alveolar/bronchiolar adenoma or carcinoma (mean, 22.2%; range, 14-32%). In female mice, the incidence of combined alveolar/bronchiolar adenoma or carcinoma was significantly increased in the mid- and high-dose groups [$p < 0.001$ and $p < 0.01$, respectively] and occurred with a positive trend [$p < 0.01$]. The tumor incidence in treated females exceeded the NTP historical control incidence for combined alveolar/bronchiolar carcinoma or adenoma (mean, 6.6%; range, 0-12%).

NTP (2007) concluded that there was equivocal evidence of carcinogenicity in female rats and no evidence of carcinogenicity in male rats.

The dose-response data for combined alveolar/bronchiolar adenoma or carcinoma from the NTP studies in male and female mice are presented in Table 2.

Table 2. Incidence of alveolar/bronchiolar tumors in male and female B6C3F$_1$ mice exposed to 4-methylimidazole via feed for 106 weeks (NTP, 2007).

Sex, strain, species	Concentration in feed (ppm)	Average daily dose[a] (mg/kg-day)	Alveolar/ bronchiolar adenoma or carcinoma (combined)[b]	Statistical significance[c]
Male B6C3F$_1$ Mice	0	0	9/49	p < 0.01[d]
	312	40	13/49	NS
	625	80	16/48	p = 0.073
	1250	170	22/49	p < 0.01
Female B6C3F$_1$ Mice	0	0	3/45	p < 0.01[d]
	312	40	8/48	NS
	625	80	17/48	p < 0.001
	1250	170	14/46	p < 0.01

[a] As reported by NTP (2007) and described in the Appendix.

[b] The denominator represents the number of mice alive at the time of the appearance of the first alveolar/bronchiolar adenoma or carcinoma (513 days in male mice and 632 days in female mice).

[c] Results of pairwise comparison using Fisher's Exact Test. 'NS' is not significant.

[d] Exact trend test p-values.

APPROACH TO DOSE-RESPONSE ANALYSIS

This section briefly reviews the genotoxicity data and other data relevant to possible mechanisms of 4-methylimidazole carcinogenicity for the purpose of determining the most appropriate approach for dose-response analysis.

NTP (2007) summarized the available data on the genotoxicity of 4-methylimidazole. 4-Methylimidazole was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA1535 with or without hamster or rat liver S9 activation enzymes. Micronuclei were not induced in bone marrow erythrocytes from male rats or male mice exposed to 4-methylimidazole via intraperitoneal injection or in peripheral blood erythrocytes from male and female mice administered the compound in dosed feed for 14 weeks.

The mechanism of carcinogenicity of 4-methylimidazole is unknown. Therefore, the default approach using a linearized multistage model is applied to derive a cancer potency estimate. The default procedures are outlined in Title 27, California Code of Regulations, section 25703. A description of the methodology used is given in the Appendix.

DOSE-RESPONSE ASSESSMENT

Animal and human cancer potency estimates were derived for 4-methylimidazole by fitting the multistage model to the dose-response data from the NTP studies in mice (Table 2). The results are summarized in Table 3 below and the derivation of the estimates is described in the Appendix. Multiplying the animal cancer potency estimate derived from each experiment by the applicable interspecies scaling factor gives an estimate of human cancer potency.

The interspecies scaling factor is derived from the ratio of body weight in humans (assumed to be 70 kilograms) to the body weight of the experimental animals (as detailed in the Appendix). The average body weights of 0.0420 kg for male mice and 0.0386 kg for female mice were calculated based on data reported by NTP (2007) for control animals in the 4-methylimidazole studies.

Male and female mice showed similar sensitivity to the carcinogenic effects of 4-methylimidazole, with male mice being slightly more sensitive. Thus, the human cancer potency estimate of 0.045 $(mg/kg\text{-}day)^{-1}$ was based on the data for male mice.

Table 3. Animal and human cancer potency estimates for 4-methylimidazole.

Sex, strain, species	Type of neoplasm	Animal cancer potency $(mg/kg\text{-}day)^{-1}$	Human cancer potency $(mg/kg\text{-}day)^{-1}$
Male B6C3F$_1$ Mice	Alveolar/bronchiolar adenoma or carcinoma (combined)	0.00376	**0.045**
Female B6C3F$_1$ Mice	Alveolar/bronchiolar adenoma or carcinoma (combined)	0.00357	0.044

Bolding indicates value selected as the basis of the NSRL.

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 0.045 $(mg/kg\text{-}day)^{-1}$, based on the combined incidence of alveolar/bronchiolar adenoma or carcinoma in male mice, was used to calculate the NSRL for 4-methylimidazole. A value of 16 µg/day was derived as shown below:

$$NSRL = \frac{10^{-5} \times 70\,kg}{0.045\,(mg/kg\text{-}day)^{-1}} \times 1000\,\mu g/mg = 16\,\mu g/day$$

REFERENCES

National Toxicology Program (NTP, 2007). *Toxicology and Carcinogenesis Studies of 4-methylimidazole (CAS No. 822-36-6) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). National Toxicology Program.* Technical Report Series No. 535. NIH Publication No. 07-4471. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, 2007.

APPENDIX: METHODOLOGY USED TO DERIVE NSRL FOR 4-METHYLIMIDAZOLE

Procedures for the development of Proposition 65 NSRLs are described in regulation in Title 27, California Code of Regulations, Sections 25701 and 25703. Consistent with these procedures, the specific methods used to derive the NSRL for 4-methylimidazole 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; U.S. Environmental Protection Agency [U.S. EPA], 2002; 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 2007 studies of 4-methylimidazole, the default 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 $q_{1(UCB)}$. When the experiment duration is at least the natural lifespan of the animals, the parameter $q_{1(UCB)}$ is taken as the animal cancer potency. When dose is expressed in units of mg/kg-day, the parameters q_1 and $q_{1(UCB)}$ 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. The risk estimate obtained is referred to by the U.S. EPA (Anderson *et al.*, 1983; U.S. EPA, 2002) as "extra risk", and is equivalent to that obtained by using the Abbott (1925) correction for background incidence.

Adjustments for experiments of short duration

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:

$$q_{animal} = q_{1(UCB)} \bullet (T/T_e)^3$$

Following Gold and Zeiger (1997) and the 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:

$$q_{animal} = q_{1(UCB)} \bullet (104/T_e)^3$$

Because the NTP (2007) studies of 4-methylimidazole were 106 weeks in duration, a correction factor to extrapolate to 104 weeks was not required and therefore $q_{animal} = q_{1(UCB)}$.

Calculation of average daily dose

NTP calculated estimated average daily dose in milligrams (mg) of 4-methylimidazole per kilograms (kg) bodyweight for each dose group based on the weights of the animals, the amount of chemical added to the feed for each dose group, and the feed consumption rates. The NTP studies lasted at least 106 weeks and the feed was available every day '*ad libitum*'. NTP (2007) reported the following estimated average daily doses: for male rats, 0, 30, 55, and 115 mg/kg; for female rats, 0, 60, 120, and 260 mg/kg; for male and female mice 0, 40, 80, and 170 mg/kg.

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 milligrams 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}) is 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-day)$^{-1}$ (see Watanabe *et al.* [1992]):

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

In the 2007 NTP studies, average body weights of 0.0420 kg for male mice and 0.0386 kg for female mice were calculated based on data reported for control animals; the default human body weight is 70 kg. An example derivation of human cancer potency using the male mouse animal cancer potency of 0.00376 (mg/kg-day)$^{-1}$ is shown below:

$$q_{human} = 0.00376 \text{ (mg/kg-day)}^{-1} \bullet (70 \text{ kg } /0.0420 \text{ kg })^{1/3} = 0.045 \text{ (mg/kg-day)}^{-1}$$

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, and q_{human} is the human cancer potency estimate.

Daily intake levels associated with lifetime cancer risks above 10^{-5} exceed the NSRL for cancer under Proposition 65 (Title 27, California Code of Regulations, section 25703). Thus for a 70 kg person, the NSRL is given by:

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

APPENDIX REFERENCES

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