NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN 2,6-XYLIDINE

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SUMMARY OF FINDINGS

The cancer potency of 2,6-xylidine was estimated from dose-response data for nasal cavity tumors (adenomas / carcinomas) in male and female CD rats exposed by diet (NTP, 1990). 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. Cancer Potency and NSRL for 2,6-Xylidine.

Chemical	Cancer Potency $(mg/kg_{bw}\text{-}day)^{-1}$	NSRL (μg/day)
2,6-Xylidine	0.0063	110

INTRODUCTION

This report describes the derivation of a cancer potency value and no significant risk level for 2,6-xylidine (CAS number 87-62-7, molecular weight 121.2). "2,6-Xylidine" was listed on July 1, 1991 as a chemical known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). 2,6-Xylidine is a chemical intermediate that is used in the production of dyes, pesticides, pharmaceuticals, fragrances and other products (NTP, 1990; IARC, 1993). It is a component of tobacco smoke and a degradation product of drugs, *e.g*., the antidiarrheal agent lidamidine hydrochloride and the local anesthetic, lidocaine (NTP, 1990; IARC, 1993). A frequently used synonym for 2,6-xylidine is 2,6-dimethylaniline.

In this document studies available for cancer dose-response assessment are provided. A summary of the derivation of the cancer potency and NSRL are also presented. Descriptions of the methodology used in the derivations are provided in the Appendix.

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

NTP (1990) conducted studies in which 2,6-xylidine was administered in the diet to rats of both sexes before breeding, through pregnancy, and through the lactation period and to the male and female offspring through their lifetime . These rat bioassays were the only available studies for estimating cancer potency. The carcinogenic responses of the male and female offspring were similar and are used in the derivation of the cancer potency and NSRL.

Groups of 56 male and 56 female Charles River CD rats (Sprague-Dawley-derived) were fed diet containing 2,6-xylidine continuously at 0, 300, 1000, or 3000 ppm for 102 weeks. The rats were the offspring of parents who were fed diets containing these same levels of 2,6-xylidine before breeding, through pregnancy, and through the lactation period. Survival of treated male offspring decreased significantly with increasing 2,6-xylidine exposure level. Survival of treated female offspring did not differ significantly from controls. Significant increases in the incidences of nasal cavity adenomas, carcinomas and adenocarcinomas were observed in males and females. Rhabdomyosarcomas, rare malignant tumors that had not been reported in this rat strain in previous studies, were observed in the nasal cavities of two high-dose male and two high-dose female rats. None were found in the untreated controls (NTP, 1990). Mixed malignant tumors with features associated with adenocarcinomas and rhabdomyosarcomas were observed in the nasal cavities of one high-dose male and one high-dose female. These unusual nasal tumors were considered to be caused by 2,6-xylidine (NTP, 1990, p.49). An undifferentiated sarcoma was also observed in the nasal cavity of one high-dose female rat. NTP noted there was an increased incidence of subcutaneous tissue fibromas and fibrosarcomas with increasing dose in males and females that "may have been related to chemical treatment." However, the nasal cavity was the primary site for 2,6-xylidine related tumors. The dose-response incidence data for male and female nasal cavity tumors are shown in Tables 2 and 3.

Table 2. Nasal Tumors in Male Charles CD Rats Administered 2,6-Xylidine in the Diet.

Concentration in Feed (ppm)	Average Daily Dose [1] (mg/kg_{bw}-day)	Tumor Incidence[2]	Statistical Significance[3]
0	0	0/56	<0.001
300	11.0	0/56	---
1000	36.8	2/56	0.3
3000	110	36/55	<0.001

[1] See Appendix for the method of average daily dose calculation.

[2] The tumor incidence is derived from Table A2 of NTP (1990) and represents combined incidence of adenoma, adenocarcinomas, carcinoma, rhabdomyosarcomas, and mixed malignant tumors of the nasal cavity. The denominator represents the number of male rats alive at the time of the appearance of the first tumor (42 weeks).

[3] Statistical significance reported by NTP (1990). The values next to treated groups are the result of pairwise comparisons by the Fisher Exact Test. The value next to the control group is the result of the Cochran-Armitage Trend Test.

Table 3. Nasal Tumors in Female Charles CD Rats Administered 2,6-Xylidine in the Diet.

Concentration in Feed (ppm)	Average Daily Dose [1] (mg/kg_{bw}-day)	Tumor Incidence[2]	Statistical Significance[3]
0	0	0/55	<0.001
300	13.3	0/55	---
1000	44.2	2/56	0.3
3000	132	32/56	<0.001

[1] See Appendix for the method of average daily dose calculation.
[2] The tumor incidence is derived from Table B2 of NTP (1990) and represents combined incidence of adenoma, papillary adenoma, adenoacarcinoma, rhabdomyosarcomas, malignant mixed tumors, and undifferentiated sarcoma of the nasal cavity. The denominator represents the number of female rats alive at the time of the appearance of the first tumor (59 weeks).
[3] Statistical significance reported by NTP (1990). The values next to treated groups are the result of pairwise comparisons by the Fisher Exact Test. The value next to the control group is the result of the Cochran-Armitage Trend Test.

APPROACH TO DOSE-RESPONSE ANALYSIS

In one report, 2,6-xylidine was not mutagenic in *Salmonella typhimurium* (TA100) in the presence of cytochrome P450-dependent metabolic activation (Zimmer *et al.*, 1980). However, Kugler-Steigmeier (1989) commented that mutagenicity to *Salmonella typhimurium* increased when the aroclor-induced rat liver extract (S9) was raised from 10 to 20 percent. Zeiger *et al.* (1988) reported weak or questionable mutagenicity of 2,6-xylidine in *Salmonella typhimurium* (TA100) in the presence of 5 or 10% Aroclor-induced hamster liver S9, but positive mutagenicity when the S9 content was increased to 30%. No evidence was found for unscheduled DNA synthesis in mouse liver in an *in vivo-in vitro* test system (Mirsalis *et al.*, 1989) or for induction of micronuclei in mouse bone marrow cells (Parton *et al.*, 1988; Parton *et al.*, 1990).

Galloway *et al*. (1987) reported that 2,6-xylidine induced chromosomal aberrations and sister chromatid exchange in Chinese hamster ovary cells *in vitro*. Mice exposed by gavage to one-half the lethal dose (LD_{50}) exhibited DNA damage in skin, urinary bladder, lung and brain (Sasaki *et al.*, 1999). Short *et al*. (1989) reported the covalent binding of 2,6-xylidine to the DNA of nasal tissue in rats exposed by gavage at a level of one-quarter the LD_{50}, provided they were first pretreated with 2,6-xylidine for nine days. Four different adducts were identified from an *in vitro* reaction between 2,6-xylidine and salmon testes DNA (Goncalves *et al.*, 2001).

2,6-Xylidine is a primary arylamine. Primary arylamines are metabolized to reactive arylhydroxylamines which can undergo further bioactivation and conjugation reactions (Damani, 1982). These reactions include those catalyzed by cytochrome P450-dependent monooxygenases and other enzymes; the free reactive hydroxylamine may be regenerated from the conjugated form in an acidic environment (Damani, 1982**;** Nelson, 1985). Ring hydroxylation may occur but to a more limited extent where ortho substituents (*e.g*., 2,6-xylidine) are present (Damani, 1982). Lindstrom *et al.* (1963) reported the presence of 4-hydroxyl-2,6-xylidine in the

urine of rats receiving 2,6-xylidine by gavage over an eight day period. Although a detailed metabolic profile of 2,6-xylidine has not been described (see Lindstrom *et al.*, 1963), Nohmi *et al*. (1983) reported the presence of the mutagenic N-hydroxylated metabolite of 2,4-xylidine in an *in vitro* rat liver microsomal system (S9) in which the yield of the mutagenic metabolite increased with increasing S9 content. Beland *et al*. (1997) reported the mutagenicity of the N-hydroxylated metabolite of 2,6-xylidine towards *Salmonella typhimurium* (TA100). Among the four DNA adducts identified from the reaction between 2,6-xylidine and salmon testes DNA, three were adducted on the carbon at position four and one on the exocyclic amino group (Goncalves *et al.*, 2001). These results may explain why increasing the concentration of liver extract produces positive mutagenicity results in *Salmonella typhimurium* (Zeigler *et al.*, 1988; Kugler-Steigmeier *et al.*, 1989).

The genotoxicity and metabolism data strongly suggest the mutagenicity of 2,6-xylidine is based on its DNA reactivity. The inconsistent genotoxicity results in *Salmonella* assays may be explained by the absence of necessary metabolic components needed to activate 2,6-xylidine. Hence, a genotoxic mechanism of carcinogenicity is likely for 2,6-xylidine.

2,6-Xylidine is a metabolite of certain drugs (*e.g.*, lidocaine) and pesticides (*e.g.*, metalaxyl (in which it is also a contaminant (Dureja *et al.*, 2000))), and is found in cigarette smoke (Puente and Josephy, 2001). Both humans and cows treated with lidocaine were found to have 2,6-xylidine in their milk (Puente and Josephy, 2001). Few data are available on the pharmaco- or toxico-kinetics of 2,6-xylidine, and none are suitable for pharmacokinetic adjustments to the dose for the extrapolation of risks to humans. The available data suggest that genotoxicity is likely involved in 2,6-xylidine carcinogenicity. Therefore, the default approach (*i.e.*, a linearized multistage model and interspecies scaling) has been applied. The approach is described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

Male and female rats were similarly sensitive to 2,6-xylidine induced nasal cavity tumors (NTP, 1990). Cancer potency estimates were derived for 2-6-xylidine based on dietary levels, using the nasal cavity tumor incidence data for males and females summarized in Tables 2 and 3 (1990). The cancer response in the more sensitive sex, female rats, produced a cancer potency estimate of 0.0063 $(mg/kg\text{-}day)^{-1}$. This estimate included adjustments for the less than lifetime study duration (102 versus 104 weeks) and rat-human differences in body size (Table 4).

Table 4. Human Cancer Potency Estimates for 2,6-Xylidine.

Basis	Cancer Potency Estimate $(mg/kg_{bw}\text{-}day)^{-1}$
Male rats	0.0056
Female rats	0.0063

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 0.0063 $(mg/kg_{bw}\text{-}day)^{-1}$, based on nasal cavity tumors in female rats, was used to calculate the NSRL for 2,6-xylidine (110 μg/day).

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR 2,6-XYLIDINE

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 2,6-xylidine 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

The lifetime average dose in units of mg/kg_{bw}-day was calculated for males and females for each of the relevant dose groups, based on the dose level, duration and regimen described in the

experiments above. The dose in mg/kg_{bw}-day was obtained from the level of 2,6-xylidine in the feed, expressed as ppm. This level was multiplied by the daily food consumption rate per body weight. The daily food consumption rates (23.3 gm/day for males and 16.9 gm/day for females) and the average body weights (0.634 kg for males and 0.383 kg for females) were obtained from the data in Tables 9 and 10 of NTP (1990).

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

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

A.3 Risk-Specific Intake Level Calculation

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

$$I = \frac{R \times bw_h}{q_{human}} \tag{5}$$

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:

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

APPENDIX REFERENCES

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