NO SIGNIFICANT RISK LEVELS (NSRLS) FOR THE PROPOSITION 65 CARCINOGENS 3,3'-DIMETHOXYBENZIDINE AND 3,3'-DIMETHOXYBENZIDINE DIHYDROCHLORIDE

August 2002

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SUMMARY OF FINDINGS

The cancer potencies of 3,3'-dimethoxybenzidine dihydrochloride (commonly called *o*-dianisidine) and 3,3'-dimethoxybenzidine dihydrochloride were estimated from dose-response data for multiple treatment-responding tumor sites among male rats exposed orally to 3,3'-dimethoxybenzidine dihydrochloride via drinking water (NTP, 1990). These sites were skin, Zymbal's gland, preputial gland, small intestine, large intestine, liver, oral cavity, lung, and brain. For each of the tumor sites listed above, a probability distribution of cancer potency estimates was derived using likelihood theory. The linear term (q_1) of the multistage model fit to dose response data for a given site represents the cancer potency for that site. The cancer potencies for the affected sites were summed probabilistically, according to their distributions, to obtain a combined distribution. This combined distribution representing cancer potency for all selected sites affected by 3,3'-dimethoxybenzidine dihydrochloride was derived through Monte Carlo analysis. The upper 95 percent confidence bound indicated by the combined distribution for these treatment-related tumor sites was taken as the cancer potency for 3,3'-dimethoxybenzidine dihydrochloride.

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 obtained from studies of 3,3'-dimethoxybenzidine dihydrochloride was used as the basis for the 3,3'-dimethoxybenzidine cancer potency after adjusting for differences in molecular weight. Cancer potency estimates and the corresponding NSRLs are given in Table 1.

Table 1. Cancer Potencies and NSRLs for 3,3'-Dimethoxybenzidine and Its Dihydrochloride.

Chemical	Cancer Potency (mg/kg-day)$^{-1}$	NSRL (µg/day)
3,3'-Dimethoxybenzidine	4.8	0.15
3,3'-Dimethoxybenzidine dihydrochloride	3.7	0.19

INTRODUCTION

This report describes the derivation of cancer potency values and no significant risk levels (NSRLs) for 3,3'-dimethoxybenzidine (CAS # 119-90-4, molecular weight 244.3) and 3,3'-dimethoxybenzidine dihydrochloride (CAS # 20325-40-0, molecular weight 317.2). "3,3'-Dimethoxybenzidine" was listed on January 1, 1988 and "3,3'-dimethoxybenzidine dihydrochloride" was listed on October 1, 1990, as chemicals known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.).

3,3'-Dimethoxybenzidine and 3,3'-dimethoxybenzidine dihydrochloride are used principally as an intermediate in the production of dyes (IARC, 1974; NTP, 1990).

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

STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT

Humans

Four human studies of dye-exposed workers were located (Frumin *et al.*, 1990; Naito *et al.*, 1995; Ouellet-Hellstrom and Rench, 1996; Hamasaki *et al.*, 1996). None of the available human studies provided sufficient information to form the basis of a potency estimate. These studies are briefly described here.

Frumin *et al.* (1990) described a case report series of six cases of bladder cancer among men of the Amalgamated Clothing and Textile Workers Union Dyers local of New York, North Carolina, and New Jersey. Significant exposure to 3,3'-dimethoxybenzidine and to 3,3'-dimethoxybenzidine were noted in several cases. The average latency from onset of exposure was 23.3 years. The total number of workers at risk was not reported, nor were the estimates of number of bladder cancers expected.

Naito *et al.* (1995) published the findings of a retrospective cohort study of 442 Japanese workers (437 men and five women) with documented exposure to one or more of the following aromatic amines (benzidine, β-naphthylamine, α-naphthylamine, or 3,3'-dimethoxybenzidine) during 1935 to 1988. The cohort was followed until 1992. Analysis of age-specific cancer mortality rates indicated a large increase in bladder carcinoma among dyestuff workers

compared to the general population. Most workers were exposed to primarily benzidine (266), however, only three of the workers were exposed exclusively to 3,3'-dimethoxybenzidine, and an additional 16 workers were exposed to 3,3'-dimethoxybenzidine and one or more other aromatic amines. The small numbers of 3,3'-dimethoxybenzidine-exposed workers prevented the authors from drawing any conclusions regarding the carcinogenicity of 3,3'-dimethoxybenzidine. Also, exposure estimates were reported as "duration of exposure", no quantitative estimates of dye concentrations were provided.

Ouellet-Hellstrom and Rench (1996) conducted a retrospective cohort study of 700 chemical workers in Connecticut between 1965 and 1989 who worked in a factory that produced a variety of chemicals, including arylamines such as dichlorobenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dimethylbenzidine. Production of benzidine had ceased prior to 1965, and only workers who had no prior exposure to benzidine were enrolled in the cohort. For the cohort an overall standardized incidence ratio for bladder cancer was reported to be 8.3 (95% CI 3.3-17.0). An exposure classification system (*e.g.*, based on factors such as job title and duration of exposure) was developed to classify workers in low, medium and high exposure categories. An increasing dose-response trend was observed for the low, medium and high exposure groups, respectively. No quantitative estimates of dye concentrations were provided, making potency estimations from this data problematic.

Hamasaki *et al.* (1996) reported on a series of cases of cancer among 438 Japanese workers who produced aromatic amines (benzidine sulfate, β-naphthylamine, α-naphthylamine, and 3,3'-dimethoxybenzidine), 88 new cases of uroepithelial cancer occurred between 1949 and 1995. The incidence rate for this cancer was 20.1%, and the average length of exposure for these cases was 7.4 years. Of the 88 cases, most were bladder cancer with the rest being cancers of the upper urinary tract. No quantitative exposure estimates of dye concentrations were provided.

Animals

Six series of animal cancer studies of 3,3'-dimethoxybenzidine or its dihydrochloride were located in the literature (Pliss, 1963; 1965; Saffiotti *et al.*, 1967; Hadidian *et al.*, 1968; Sellakumar *et al.*, 1969; Pliss and Volfson, 1979a,b; Schieferstein *et al.*, 1990; NTP, 1990). Early studies conducted by the oral route (feed) showed that 3,3'-dimethoxybenzidine or 3,3'-dimethoxybenzidine dihydrochloride induced neoplasia in rats and hamsters (Pliss, 1963; Pliss, 1965; Saffiotti *et al.*, 1967; Sellakumar *et al.*, 1969; Hadidian *et al.*, 1968; Pliss and Volfson, 1979a,b). These earlier studies are the less suitable for potency evaluation due to the small numbers of animals used, the instability of the chemical in feed, and poor survival. Schieferstein *et al.* (1990) exposed BALB/c mice to 3,3'-dimethoxybenzidine dihydrochloride in their drinking water for up to 112 weeks. No treatment related increases in neoplasia were observed. NTP (1990) conducted studies in which 3,3'-dimethoxybenzidine dihydrochloride was administered by the oral route (drinking water) to male and female F344/N rats and observed increased incidences of tumors at multiple sites. NTP determined that the chemical is stable in drinking water, performed range-finding studies to select appropriate doses, used 50 animals per dose group, performed complete histopathology and provided thorough reporting of the study. For these reasons, the NTP studies in rats are the most appropriate for estimating a cancer potency for the purposes of Proposition 65. The details of the study are as follows.

In the NTP (1990) bioassays, groups of male and female F344/N rats were dosed via drinking water with 3,3'-dimethoxybenzidine dihydrochloride continuously at 0, 80, 170, or 330 ppm for 21 months. NTP (1990) estimated that the average daily doses over the study duration were 0, 6, 12, or 21 $(mg/kg-d)^{-1}$ for males and 0, 7, 14, or 23 $(mg/kg-d)^{-1}$ for females, respectively. The survival of all dosed groups was significantly lower than that of controls, animals died as early as day 401 for high dose males and day 304 for high dose females. The reduced survival was primarily due to neoplasia.

In male rats, the NTP reported significant increases with increasing dose in the incidences of skin basal cell and sebaceous gland carcinomas or adenomas (combined), skin squamous cell or carcinomas adenomas (combined), Zymbal's gland carcinomas or adenomas (combined), preputial gland carcinomas or adenomas (combined), oral cavity squamous cell carcinomas or papillomas (combined), small intestine adenocarcinomas, large and small intestine adenocarcinomas or adenomatous polyps (combined), liver hepatocellular carcinomas or neoplastic nodules (combined), and mesotheliomas. NTP also attributed the increase in brain astrocytomas, a rare tumor, in the dosed groups to exposure to 3,3'-dimethoxybenzidine dihydrochloride. Incidences of these tumors are presented in Table 2.

Table 2. Incidences of Neoplastic Lesions Among Male F344/N Rats Treated with 3,3'-Dimethoxybenzidine Dihydrochloride in Drinking Water for 21 Months[1].

Tumor Site	Average Dose[2] (mg/kg-day)				Trend[3]
	0	6	12	21	
Skin basal cell or sebaceous gland carcinomas or adenomas	2/59	33/44	56/72	41/56	p < 0.001
Skin squamous cell carcinomas or papillomas	0/59	13/42	28/65	22/48	p < 0.001
Zymbal's gland carcinomas or adenomas	0/58	10/45	25/75	30/60	p < 0.001
Preputial gland carcinomas or adenomas	16/59	12/42	33/73	29/59	p = 0.0026
Oral cavity squamous cell carcinomas or papillomas	1/59	8/44	10/73	11/57	p = 0.0072
Small intestine adenocarcinomas	0/59	4/44	7/75	5/60	p = 0.053
Large intestine adenocarcinomas or adenomatous polyps	0/59	1/44	8/73	8/57	p < 0.001
Liver hepatocellular carcinomas or neoplastic nodules	1/58	4/39	7/54	8/35	p < 0.001
Mesothelioma	2/59	1/44	7/72	6/56	p = 0.033
Brain astrocytomas	0/58	2/37	3/48	1/30	p = 0.16

[1] Incidence data shown are based on effective rates: the number of animals with tumor/effective number of animals (*i.e.*, number of animals alive at first occurrence of tumor in any group). Rates obtained from NTP (1990), Table A3.
[2] Average doses as reported by NTP (1990).
[3] Exact trend test

In female rats, the NTP reported the following tumors related to treatment: skin basal cell carcinomas or adenomas, Zymbal's gland carcinomas or adenomas, clitoral gland carcinomas or adenomas (combined), large intestine adenocarcinomas or adenomatous polyps (combined), liver hepatocellular carcinomas or neoplastic nodules (combined), oral cavity squamous cell carcinomas or papillomas (combined), mammary gland adenocarcinomas, and uterus/cervix carcinomas or adenomas (combined). NTP noted that the increases neoplasms of the skin, oral cavity, intestine, liver and uterus/cervix were only marginally increased, but considered these neoplasms compound-related because of the low spontaneous incidence of these neoplasms in female F344/N rats and the chemically-related early deaths.

Table 3. Incidences of Neoplastic Lesions Among Female F344/N Rats Treated with 3,3'-Dimethoxybenzidine Dihydrochloride in Drinking Water for 21 Months[1].

Tumor site	Average Dose[2] (mg/kg-day)				Trend[3]
	0	7	14	23	
Clitoral gland carcinomas or adenomas	7/58	27/44	48/74	41/55	p < 0.001
Zymbal's gland carcinomas or adenomas	1/60	12/45	21/74	16/59	p < 0.001
Mammary gland adenocarcinomas	1/60	2/45	14/73	20/57	p < 0.001
Skin basal cell carcinomas or adenomas	0/59	4/44	3/48	2/35	p = 0.13
Oral cavity squamous cell carcinomas or papillomas	2/60	2/45	6/68	5/52	p = 0.069
Large intestine adenocarcinomas or adenomatous polyps	0/59	1/44	1/48	3/35	p = 0.017
Hepatocellular carcinomas or neoplastic nodules	0/59	1/44	0/47	3/38	p = 0.017
Uterus/cervix adenoma or carcinoma	0/59	4/44	2/48	2/35	p = 0.16

[1] Incidence data shown are based on effective rates: the number of animals with tumor/effective number of animals (*i.e.*, number of animals alive at first occurrence of tumor in any group). Rates obtained from NTP (1990), Table B3.
[2] Average doses as reported by NTP (1990).
[3] Exact trend test.

APPROACH TO DOSE RESPONSE ANALYSIS

3,3'-Dimethoxybenzidine was mutagenic in *Salmonella* and induced unscheduled DNA synthesis in rat hepatocyte primary cultures (NTP, 1990). 3,3'-Dimethoxybenzidine dihydrochloride was mutagenic in *Salmonella* and induced sister chromatid exchange and chromosomal aberrations in mammalian cells *in vitro* (NTP, 1990). These findings, coupled with the observed mutagenic activity of several metabolites of 3,3'-dimethoxybenzidine and other structurally similar compounds (NTP, 1990) are strongly suggestive that a genotoxic mode of action is operative. Given this information on mode of action for 3,3'-dimethoxybenzidine and the dihydrochloride, a linear multistage model was used to estimate the slope of the dose-response in the low dose region.

In the rat bioassays (NTP, 1990), treatment resulted in significant early mortality, which was primarily due to cancers at different sites. The multistage model did not fit the tumor data well for skin basal cell or sebaceous gland carcinomas and adenomas (combined) in males and clitoral gland carcinomas and adenomas (combined) and Zymbal's gland carcinomas and adenomas (combined) in females, apparently due to increases in competing causes of death with increasing dose. As described in more detail below and in the Appendix, standard procedures (Anderson *et*

al., 1983) that partially address the potential underestimation of potency due to early death were applied in modeling the incidence data for male skin tumors and female clitoral gland and Zymbal's gland tumors.

Since 3,3'-dimethoxybenzidine dihydrochloride induced tumors at multiple sites in both male and female rats, cancer potency estimates based on a single tumor site will not fully represent the overall cancer risks due to exposure to the compound; thus, a combined cancer potency estimate for treatment related cancer sites was derived for male rats (the more sensitive sex) using Monte Carlo analysis (see below).

There are insufficient data to support dose adjustments based on pharmacokinetic models. Therefore, the default approach (*i.e.*, a linearized multistage model, adjustments for less than lifetime study duration, and interspecies scaling) has been applied. The approach used is described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

Cancer potency estimates for each tumor site for male and female rats treated orally with 3,3'-dimethoxybenzidine dihydrochloride were derived from the NTP studies described above (NTP, 1990), using methods described in the Appendix. The cancer potency estimates are summarized in Table 4. Potency estimates indicate that male rats are more sensitive to tumor induction by 3,3'-dimethoxybenzidine dihydrochloride than female rats, but details on the calculations for both males and females are provided below.

The multistage model provides adequate statistical fit for all the datasets, except for the tumor incidence of male rat skin basal cell or sebaceous gland carcinomas and adenomas (combined) (Table 2), female rat clitoral gland carcinomas and adenomas (combined), and female rat Zymbal's gland carcinomas and adenomas (combined) (Table 3). The tumor responses in male rat skin in the low-, mid- and high-dose groups were all high (ranging from about 70 to 80 percent), resulting in a dose-response curve that is supralinear (Figure 1). The shape of this dose-response curve likely reflects the high level of responsiveness of this tissue type in combination with the reduced survival of the higher dose groups. Modeling all dose groups, the multistage model provided poor fit (chi-squared test, p<0.001). Following the U.S. Environmental Protection Agency (U.S. EPA) procedures described in Anderson *et al*. (1983), when the multistage model does not fit the data adequately, data at the highest dose are deleted, and the model fitted to the remaining data. This is repeated until an acceptable fit is obtained, as measured by the chi-squared goodness-of-fit test. In the case of male rat skin tumors, removal of the high-dose group improved the fit slightly, but the goodness-of-fit was still inadequate (chi-squared test, p=0.01). For this reason, both the mid- and high-dose groups were removed, and a cancer potency slope for male rat skin tumors was estimated from the control and low-dose groups. For female rat clitoral gland and Zymbal's gland tumors, removal of the top dose groups, respectively, resulted in adequate goodness-of-fit of the multistage model.

Figure 1. Skin Basal Cell and Sebaceous Gland Carcinomas and Adenomas (Combined) Among Male Rats Fed 3,3'-Dimethoxybenzidine Dihydrochloride in the Diet for 21 Months.
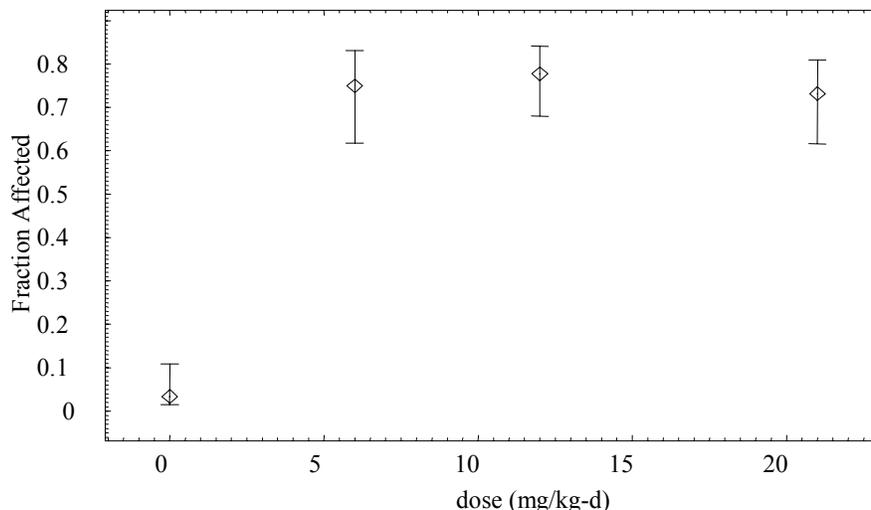
Since 3,3'-dimethoxybenzidine dihydrochloride induced tumors at multiple sites in male rats, a combined potency estimate for all treatment-related tumor sites was derived using Monte Carlo analysis. For each tumor site, a distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model was generated with the MSTAGE computer program (Crouch, 1998), which had been modified to tabulate percentile values. A combined distribution (Figure 2) was created by adding q_1 for each tumor site, according to its distribution, through one million Monte Carlo trial simulations (Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound of the combined distribution was taken as the basis of the cancer potency estimate for the combined tumor sites (Table 4).

For male rats, distributions of the cancer potency estimates were combined for the following tumor sites: skin basal cell and sebaceous gland carcinomas or adenomas (combined, top- and mid-dose groups removed), skin squamous cell or carcinomas adenomas (combined), Zymbal's gland carcinomas or adenomas (combined), preputial gland carcinomas or adenomas (combined), oral cavity squamous cell carcinomas or papillomas (combined), large and small intestine adenocarcinomas or adenomatous polyps (combined), hepatocellular carcinomas or neoplastic nodules (combined), mesotheliomas and brain astrocytomas. The combined distribution of animal cancer potency estimates for 3,3'-dimethoxybenzidine dihydrochloride in male rats is shown in Figure 2.

Figure 2. Combined Distribution of Potency Estimates (mg/kg-day)$^{-1}$ for All 3,3'-Dimethoxybenzidine Dihydrochloride-Related Tumor Sites Among Male Rats[1].
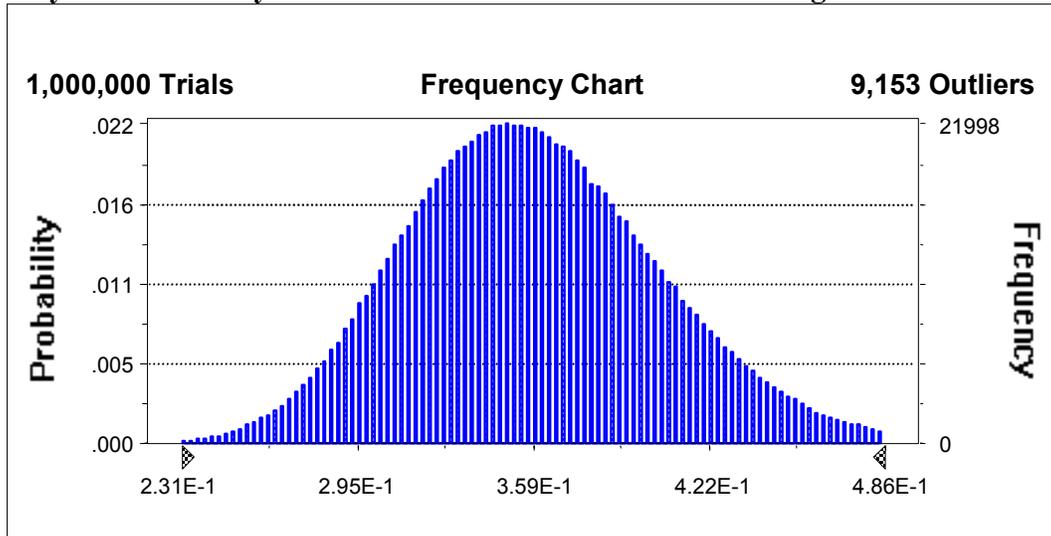
^{1} The 95 percent upper confidence bound of this distribution, 0.441 (mg/kg-d)$^{-1}$, represents the 91-week animal cancer potency for all treatment-related tumors in male rats. This corresponds to a lifetime animal potency of 0.658 (mg/kg-d)$^{-1}$ and a human potency estimate of 3.7 (mg/kg-d)$^{-1}$ (see Appendix).

Using methods described in the Appendix, animal potencies derived from the 91-week study are projected to estimates of potencies associated with lifetime exposures. These lifetime values are then used to predict human cancer potency. Table 4 provides human potency estimates derived from the individual treatment-related sites in the male and female bioassays on the compound (NTP, 1990), as well as the combined estimate for the most sensitive sex. Because the values given in Table 4 are derived from the 95 percent upper confidence bounds, the sum of the values for the individual sites does not correspond to the combined estimate. The estimate for all treatment-related cancers corresponds to the upper 95 percent confidence bound on the distribution obtained through the Monte Carlo simulation (Figure 2).

Table 4. Human Cancer Potency Estimates for 3,3'-Dimethoxybenzidine Dihydrochloride.

Tumor site	Cancer Potency $(mg/kg\text{-}day)^{-1}$	
	Males	Females
Clitoral gland carcinomas or adenomas High-dose group removed	--- ---	0.73 0.89

Skin basal cell carcinomas or adenomas
  High-dose group removed
  Mid- and high-dose groups removed
	1.0
	1.1
	2.6
		0.079
		NA
		NA

Skin squamous cell carcinomas or papillomas	0.41	---
Zymbal's gland carcinomas or adenomas High-dose group removed	0.35 NA	0.23 0.33
Mammary gland adenocarcinomas	---	0.15
Oral cavity squamous cell carcinomas or papillomas	0.14	0.06
Preputial gland carcinomas or adenomas	0.25	---
Large intestine adenocarcinomas or adenomatous polyps	0.094	0.049
Small intestine adenocarcinomas or adenomatous polyps	0.085	---
Liver hepatocellular carcinomas or neoplastic nodules	0.14	0.039
Mesothelioma	0.067	---
Brain astrocytomas	0.066	---
Uterus/cervix carcinomas or adenomas	---	0.072
All 3,3'-dimethoxybenzidine dihydrochloride-related tumor sites	**3.7**	**---**

  Bolding indicates values selected as the basis of the NSRL.
  NA = not applicable.

The cancer potency estimate for 3,3'-dimethoxybenzidine was derived by multiplying the estimate for 3,3'-dimethoxybenzidine dihydrochloride for male rats (the most sensitive sex) by the molecular weight of the dihydrochloride divided by the molecular weight of 3,3'-dimethoxybenzidine (*i.e.*, 317.2/244.3). The resulting cancer potency is 4.8 $(mg/kg\text{-}d)^{-1}$.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The cancer potency estimates derived above were used to calculate NSRLs for 3,3'-

dimethoxybenzidine (0.15 µg/day) and 3,3'-dimethoxybenzidine dihydrochloride (0.19 µg/day), after rounding to two significant figures.

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APPENDIX: METHODOLOGY USED TO DERIVE RISK-SPECIFIC INTAKE LEVELS FOR 3,3'-DIMETHOXYBENZIDINE AND 3,3'-DIMETHOXYBENZIDINE DIHYDRO-CHLORIDE

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 NSRLs for 3,3'-dimethoxybenzidine and 3,3'-dimethoxybenzidine dihydrochloride are outlined in this Appendix.

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, 1996; Anderson *et al.*, 1983):

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

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 $(mg/kg\text{-}day)^{-1}$. Details of the estimation procedure are given in Crump (1981) and Crump *et al.* (1977).

Due to cancer related mortality, the dose-response data for the skin tumors in male rats (NTP, 1990) were highly supralinear (*i.e.*, the trend in tumor incidence is less than linear with increasing dose). In females, the multistage model provided adequate statistical fit for all sites except clitoral gland carcinomas and adenomas (combined), and Zymbal's gland carcinomas and adenomas (combined). Following the U.S. Environmental Protection Agency (U.S. EPA) procedures described in Anderson *et al.* (1983), whenever the multistage model does not fit the data adequately, data at the highest dose are deleted and the model fitted to the remaining data. This is repeated until an acceptable fit is obtained, as measured by the chi-square goodness-of fit test. For analysis of the male skin tumor data, the mid- and high-dose groups were dropped. For analyses of tumor data for the female clitoral gland and Zymbal's gland, the high-dose group was dropped.

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:

$$q_{animal} = q_1* \cdot (T/T_e)^3 \qquad (2)$$

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:

$$q_{animal} = q_1* \cdot (104/T_e)^3 \qquad (3)$$

Potencies from the 21-month (91 week) rat bioassays (NTP, 1990) were adjusted to reflect the projected response from lifetime exposure (for the rat, assumed to be 104 weeks), *i.e.*, $q_1* \cdot (104/91)^3$.

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-day was calculated by NTP (1990) for each of the relevant dose groups, based on the dose level, duration and regimen described in the experiments above. NTP (1990) dosed groups of male and female F344/N rats were dosed via drinking water with 3,3'-dimethoxybenzidine dihydrochloride continuously at 0, 80, 170, or 330 ppm for 21 months. NTP (1990) estimated that the average daily doses over the study duration were 0, 6, 12, or 21 $(mg/kg\text{-}d)^{-1}$ for males and 0, 7, 14, or 23 $(mg/kg\text{-}d)^{-1}$ for females, respectively.

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

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

In this calculation, body weights for male and female rats were 0.4 kg and 0.3 kg, respectively, corresponding to the average body weights of the control and dosed groups in the final months of the study (NTP, 1990).

3. Risk-Specific Intake Level Calculation

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

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

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 intake levels exceeding the no significant risk level are given by:

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

APPENDIX REFERENCES

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