NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN C.I. DIRECT BLUE 218

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SUMMARY OF FINDINGS

The human cancer potency of C.I. Direct Blue 218 was estimated using the linearized multistage model from dose-response data for hepatocellular adenomas or carcinomas (combined) in female $B6C3F_1$ mice exposed via feed (National Toxicology Program [NTP], 1994). 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 human cancer potency estimate for C.I. Direct Blue 218 is 0.014 $(mg/kg\text{-}day)^{-1}$and the corresponding NSRL is 50 μg/day.

Table 1. Cancer Potency and NSRL for C.I. Direct Blue 218.

Chemical	Cancer potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
C.I. Direct Blue 218	0.014	50

INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for C.I. Direct Blue 218 (CAS number 28407-37-6). C.I. Direct Blue 218 was listed on August 26, 1997 as a chemical 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*.). The compound is a copper complex of 3,3′ - [(3,3′ -dihydroxy[1,1′ -biphenyl]-4,4′ -diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, used to dye a variety of materials including paper and textile goods (NTP, 1994). C.I. Direct Blue 218, which is a benzidine-based dye, has many synonyms and common trade names (NTP, 1994). The U.S. production/import volume was reported to be between 10,000 and 500,000 pounds in 2002 (U.S. EPA, 2002).

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 C.I. Direct Blue 218. Increases in urinary bladder neoplasms in humans exposed to other benzidine-based dyes have been observed in epidemiological studies.

The only cancer bioassays available for estimating the potency of C.I. Direct Blue 218 were conducted by NTP (1994), and are discussed in detail below. The NTP studies on C.I. Direct Blue 218 were part of NTP's Benzidine Dye Initiative, which examined five representative benzidine congeners for toxicity and carcinogenicity.

NTP (1994) exposed groups of 60 male and 60 female F344/N rats and $B6C3F_1$ mice to C.I. Direct Blue 218 in their feed at 0, 1,000, 3,000 or 10,000 ppm C.I. Direct Blue 218 for 105 weeks. Ten animals of each dose group for each species/sex were sacrificed at 15 months.

NTP found some evidence of neoplastic effects in male rats based on the observation of pharyngeal neoplasms. There were six animals with tumors, all found in the high dose group. No treatment related tumors were observed in female rats and no tumors were observed in the interim sacrifice group of either sex.

NTP found clear evidence of carcinogenicity in male and female mice, based on significant, dose-related increases in the incidences of hepatocellular adenomas and carcinomas. A few tumors were also observed in the interim sacrifice group. The incidences in the highest dose groups for both males and females were much higher than the historical incidence of these neoplasms in untreated controls (NTP, 1994). NTP also found some kidney and small intestine neoplasms in male mice that may have been related to exposure to C.I. Direct Blue 218. Male and female mice were the more sensitive animals in the NTP bioassays; the data from the studies in mice are discussed and analyzed further below. The data from the interim sacrifice groups were not included in the current analysis because of the shorter experiment time.

There was no difference in survival rates of dosed mice compared to mice in the control group (NTP, 1994). Male and female mice in the highest dose group (10,000 ppm) weighed 10% and 29% lower, respectively, than the controls throughout most of the study and finished the study weighing 19% and 27%, respectively, below controls. Male and female mice in the 3,000 ppm group weighed 1% to 10% lower than controls. However, dosed and control mice had similar feed consumption throughout the study and there were no clinical signs of toxicity associated with C.I. Direct Blue 218 (NTP, 1994).

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

Table 2. Incidences of Hepatocellular Tumors in Male and Female B6C3F$_1$ Mice Exposed to C.I. Direct 218 via Feed for 105 Weeks.

Sex, strain, species	Concentration in feed (ppm)	Average daily dose[1] (mg/kg-day)	Hepatocellular adenoma or carcinoma (combined)[2]	Statistical significance
Male B6C3F$_1$ Mice	0	0	21/48	$p < 0.001$[3]
	1,000	120	20/49	$p = 0.465$N[4]
	3,000	360	23/49	$p = 0.456$[4]
	10,000	1,520	45/50	$p < 0.0001$[4]
Female B6C3F$_1$ Mice	0	0	10/45	$p < 0.001$[3]
	1,000	140	15/49	$p = 0.247$[4]
	3,000	470	21/48	$p < 0.05$[4]
	10,000	2,050	45/45	$p < 0.0001$[4]

[1] Average daily dose as reported by NTP (1994).

[2] The denominator represents the number of mice alive at the time of the appearance of the first hepatocellular adenoma or carcinoma (519 days in male mice, and 541 days in female mice).

[3] Trend test *p*-values as reported by NTP (1994) based on the Cochran-Armitage trend test.

[4] The *p*-values from pairwise comparison with controls (Fisher Exact Test). An "N" after the *p*-value signifies that the incidence in the dose group is lower than that in the control group.

APPROACH TO DOSE-RESPONSE ANALYSIS

This section reviews the genotoxicity data on C.I. Direct Blue 218 and other data relevant to possible mechanisms of carcinogenicity for the purpose of determining the most appropriate approach for dose-response analysis.

NTP (1994) reported the results from a series of genotoxicity assays on C.I. Direct Blue 218. C.I. Direct Blue 218 was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537, with or without metabolic activation. C.I. Direct Blue 218 was also negative in a modified assay in *Salmonella typhimurium* strain TA1538, which used reductive metabolism followed by oxidative metabolism. The chemical did not induce chromosomal aberrations in Chinese hamster ovary cells in the presence or absence of S9 and was negative in the sex-linked recessive lethal mutation assay in *Drosophila melanogaster*. However, NTP reported that the highest dose of C.I. Direct Blue 218 did induce "a small but significant increase in sister chromatid exchanges" in Chinese hamster ovary cells without S9 activation. OEHHA did not locate any additional genotoxicity studies in the literature.

C.I. Direct Blue 218 is a benzidine-based dye, and more specifically, a congener of 3,3´-dihydroxybenzidine. Both 3,3´-dihydroxybenzidine and benzidine are mutagenic in *S. typhimurium* with, but not without metabolic activation (NTP, 1994). Benzidine, a known human carcinogen that is genotoxic, induces liver tumors in mice, as does C.I. Direct Blue 218. NTP (1994) has noted that, "the C.I. Direct Blue 218 component responsible for producing hepatic neoplasms may be structurally related to the benzidine metabolite associated with the

induction of hepatic neoplasms." The benzidine hepatic metabolite is known to form DNA adducts and NTP (1994) hypothesized that "Metabolites of C.I. Direct Blue 218 may also form DNA adducts in the mouse liver leading to genetic alterations that would allow the cells to escape normal growth control mechanisms and result in a neoplastic response." However, the precise mechanism of carcinogenicity of C.I. Direct Blue 218 is unknown. Thus the development of a biologically based model for cancer potency estimation is not feasible. 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 default procedures outlined in Title 22, California Code of Regulations, Section 12703 are described in detail in the Appendix.

DOSE-RESPONSE ASSESSMENT

Table 3 summarizes the animal and human cancer potency estimates derived for C.I. Direct Blue 218 based on the hepatocellular tumor incidence data in mice (see Table 2). Male and female mice showed similar sensitivity to the carcinogenic effects of C.I. Direct Blue 218, with female mice being slightly more sensitive. The human cancer potency estimate of 0.014 $(mg/kg\text{-}day)^{-1}$ based on the data in female mice includes adjustments for mouse-to-human differences in body size, as detailed in the Appendix.

Table 3. Human Cancer Potency Estimates for C.I. Direct Blue 218.

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	Hepatocellular adenoma or carcinoma (combined)	0.001005	0.012
Female $B6C3F_1$ mice	Hepatocellular adenoma or carcinoma (combined)	0.001153	**0.014**

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 0.014 $(mg/kg\text{-}day)^{-1}$, based on hepatocellular adenoma or carcinoma (combined) in female mice, was used to calculate the NSRL for C.I. Direct Blue 218. A value of 50 μg/day was derived as shown below:

$$\text{NSRL} = \frac{10^{-5} \times 70\,\text{kg}}{0.014\,(\text{mg/kg - day})^{-1}} \times 1000\,\mu\text{g/mg} = 50\,\mu\text{g/day}$$

REFERENCES

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APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE THE NSRL FOR C.I. DIRECT BLUE 218

Procedures for the development of Proposition 65 NSRLs are described in regulation Title 22, California Code of Regulations, Sections 12701 and 12703). Consistent with these procedures, the specific methods used to derive the NSRL for C.I. Direct Blue 218 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):

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

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 C.I. Direct Blue 218, the default linearized multistage model defaults to three stages, or four parameters, $q_0,\ldots q_3$. The parameter q_0 represents the background lifetime incidence of the tumor. 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 $(mg/kg\text{-}day)^{-1}$. 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 the animal cancer potency (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:

$$q_{animal} = q_{1(UCB)} \bullet (T/T_e)^3 \quad (2)$$

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:

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

Because the NTP (1994) studies of C.I. Direct Blue 218 were 105 weeks in duration, a correction factor to extrapolate to 104 weeks was not required and therefore, $q_{animal} = q_{1(UCB)}$.

Calculation of the lifetime average dose

NTP calculated estimated average daily dose in mg of C.I. Direct Blue 218 per 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. NTP (1994) reported the following estimated average daily doses: for male rats, 0, 40, 120, and 440 mg/kg; for female rats, 0, 50, 140, and 470 mg/kg; for male mice, 0, 120, 360, and 1520 mg/kg; and for female mice, 0, 140, 470, and 2050 mg/kg. The NTP studies lasted at least 104 weeks and the feed was available every day '*ad libitum*', so the average daily dose is equal to the lifetime average dose.

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}) 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 $(mg/kg\text{-}day)^{-1}$ (see Watanabe *et al.* [1992]):

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

The average body weights of 0.0403 kg for male mice and 0.0390 kg for female mice were calculated based on data reported by NTP (1994) for control animals; the default human body weight is 70 kg. An example calculation using the female mice animal cancer potency of $0.001153\ (mg/kg\text{-}day)^{1}$ is shown below:

$$q_{human} = 0.001153\ (mg/kg\text{-}day)^{-1} \bullet (70\ kg\ /0.0390\ kg\ )^{1/3} = 0.014\ (mg/kg\text{-}day)^{-1} \quad (5)$$

A.3 Risk-Specific Intake Level Calculation

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

$$I = \frac{R \times bw_h}{q_{human}} \quad (6)$$

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 22 California Code of Regulations, section 12703). Thus for a 70 kg person, the NSRL is given by:

$$NSRL = \frac{10^{-5} \times 70\ kg}{q_{human}} \times 1000\ \mu g / mg. \quad (7)$$

APPENDIX REFERENCES

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