EXPEDITED CANCER POTENCY VALUES AND NO SIGNIFICANT RISK LEVELS (NSRLs) FOR FOUR PROPOSITION 65 CARCINOGENS: ISOBUTYL NITRITE, NALIDIXIC ACID, *o*-PHENYLENEDIAMINE, *o*-PHENYLENEDIAMINE HYDROCHLORIDE

August 2002

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment (OEHHA)
California Environmental Protection Agency

SUMMARY OF FINDINGS

Cancer potencies were estimated for four Proposition 65 carcinogens with dose-response data summarized by Gold and colleagues (Gold and Zeiger, 1997; Gold *et al.*, 1999) in the Carcinogenic Potency Database (CPDB) (http://potency.berkeley.edu/database.html), using an expedited methodology. The expedited approach represents the first level of a three-tiered risk assessment procedure currently in place for timely and efficient development of cancer potencies and Proposition 65 "no significant risk levels" (NSRLs) and has been shown previously to produce reliable potency values (OEHHA, 1992; Hoover *et al.*, 1995). Values generated using the expedited approach may be reevaluated if scientific considerations indicate that more detailed analysis associated with a conventional risk assessment is warranted. The chemicals assessed here are isobutyl nitrite, nalidixic acid, *o*-phenylenediamine, and *o*-phenylenediamine dihydrochloride. This report describes the methodology used and the basis for cancer potency estimation for each of these compounds. The upper 95 percent confidence bound on the linear term of the multistage model fit to cancer dose response data is taken as the estimate of cancer potency. The derivation takes into account species differences and length of the bioassay. The Proposition 65 NSRL is defined in regulation as the daily level posing a 10^{-5} lifetime risk of cancer. Cancer potency estimates and the corresponding NSRLs are given in Table 1 below.

Table 1. Cancer Potencies and NSRLs.

Chemical	CAS No.	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
Isobutyl nitrite	542-56-3	0.095	7.4
Nalidixic acid	389-08-2	0.025	28
o-Phenylenediamine	95-54-5	0.027	26
o-Phenylenediamine dihydrochloride	615-28-1	0.016	44

INTRODUCTION

This report describes the derivation of cancer potency values and "no significant risk levels" (NSRLs) for the four Proposition 65 carcinogens (California Health and Safety Code 25249.5 *et seq*.) listed in Table 1. An expedited procedure was applied in the derivation (OEHHA, 1992; Hoover *et al.*, 1995); the methodology is summarized below. The studies used as the basis of the potency derivation for each chemical, and the relevant data are described. The basis for selecting each cancer potency estimate is also discussed.

METHODOLOGY

In a typical, non-expedited assessment, a full literature search is undertaken to locate all data on the carcinogenicity and dose response characteristics of the compound. This is followed by a review of the pharmacokinetic and mechanistic (*e.g.*, genotoxicity) data, and a dose response review of all adequate bioassays. Occasionally the data support a pharmacokinetic analysis in the derivation of target dose estimates, or a dose response model different from the default. The expedited procedure differs from this usual practice in two ways. First, it relies on cancer dose response data evaluated and extracted from the original literature by Gold and colleagues (Gold and Zeiger, 1997; Gold *et al.*, 1999) and contained in the Carcinogenic Potency Database (CPDB) currently electronically available at http://potency.berkeley.edu/database.html. Second, under the expedited procedure the choice of the multistage model is automatic and pharmacokinetic adjustments are not employed. The default procedures used to derive expedited cancer potency values are specified in the administrative regulations for Proposition 65 (Title 22 California Code of Regulations [CCR] 12703) (Hoover *et al.*,1995; OEHHA, 1992). The expedited approach, which represents the first level of a three-tiered risk assessment procedure currently in place for development of cancer potencies and Proposition 65 NSRLs, has been shown to be a reliable means for generating potency values and NSRLs in a timely and efficient manner. As described in Title 22 CCR 12703, an NSRL generated using the expedited approach may be reevaluated if scientific considerations indicate that more detailed analysis associated with a conventional risk assessment is warranted.

The methods for expediting potency estimation incorporate the following assumptions:

- The dose-response relationship for carcinogenic effects in the most sensitive species tested is representative of that in humans.

- Observed experimental results can be extrapolated across species by use of the interspecies factor based on "surface area scaling."

- The dose to the tissue giving rise to a tumor is assumed to be proportional to the administered dose.

- The multistage polynomial can be used to extrapolate potency outside the range of experimental observations to yield estimates of "low" dose potency.

- Cancer hazard increases with the third power of age.

Data Set Selection: The following criteria are used for data selection:

- Data sets with statistically significant increases in cancer incidence with dose.

- When several studies are available, and one study stands out as being of higher quality due to numbers of dose groups, magnitude of the dose applied, duration of study, or other factors, the higher quality study is chosen as the basis for potency calculation.

- When there are multiple studies of similar quality in the sensitive species, the geometric mean of potencies derived from these studies is taken. If the same investigators tested both sexes of the same species/strain under the same laboratory conditions, and no other adequate studies are available for that species, the data set for the more sensitive sex is selected.

- Potency is derived from data sets that tabulate malignant tumors, combined malignant and benign tumors, or tumors that would have likely progressed to malignancy.

Mathematical Model: Cancer potency is defined as the slope of the dose response curve at low doses. Following the default approach, the Crump linearized multistage polynomial (Crump *et al*., 1977) describes the dose response relationship:

$$\text{Probability of cancer} = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + ...)] \quad (1)$$

The slope (q_1) is estimated by fitting the polynomial to dose response data collected at high doses using one of the statistical curve-fitting packages developed for this purpose [*e.g.*, "Tox_Risk" (Crump *et al*., 1993); "MSTAGE" (Crouch, 1992)]. For bioassays with exposures throughout the study period, dose (d) is the average daily dose over the experimental period. Cancer potency is estimated from the upper 95 percent confidence bound on the linear coefficient q_1, which will be termed q_1*.

For a given chemical, the model is fit to one or more data sets. As discussed in the section above, the default is to select the data for the most sensitive species and sex. When there are several bioassays of equivalent quality, a geometric mean is taken.

Standard bioassays on mice and rats last approximately two years. In standard risk assessments, this is the assumed lifespan for these species. Animals in experiments of shorter duration are at a lower risk of developing tumors than those in the standard bioassay; thus potency is underestimated unless an adjustment for experimental duration is made. In estimating potency, short duration of an experiment is taken into account by multiplying q_1^* by a correction factor equal to the cube of the ratio of the assumed standard lifespan of the animal to the duration of the experiment (T_e). This assumes that the cancer hazard would have increased with the third power of the age of the animals had they lived longer:

$$q_{animal} = q_1^* \bullet (104 \text{ weeks}/T_e)^3 \quad (2)$$

To estimate human cancer potency, q_{animal} values derived from bioassay data are multiplied by an interspecies scaling factor (K; the ratio of human body weight (bw_h) to test animal body weight (bw_a), taken to the 1/3 power; see Anderson *et al.* (1983) for details):

$$K = (bw_h/bw_a)^{1/3} \quad (3)$$

Thus,

$$\text{Cancer potency} = q_{human} = K \bullet q_{animal} \quad (4)$$

To calculate K, unless otherwise specified, default body weights of 0.5 and 0.35 kg were used for male and female rats, and 0.03 and 0.025 for male and female mice, respectively (Gold and Zeiger, 1997). Values for interspecies scaling and correction for study duration, as well as the recommended cancer potency value are reported in tabular form for each of the chemicals addressed in this report. From these human cancer potencies, exposures associated with a given level of cancer risk can be derived. For example, the NSRL for Proposition 65 is the intake (I) associated with a lifetime cancer risk of 10^{-5} or lower. For a 70-kg adult, this level is calculated according to the following equation:

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

where q_{human} is given in units of $(\text{mg/kg-day})^{-1}$ and I in units of µg/day.

DERIVATION OF HUMAN CANCER POTENCY VALUES AND NSRLs

Cancer potency estimates and NSRLs were derived for four Proposition 65 carcinogens, as described below for each chemical.

ISOBUTYL NITRITE (CAS NO. 542-56-3)

Results from the studies by NTP (1996) were listed in the CPDB. NTP exposed male and female $B6C3F_1$ mice and male and female F344 rats to isobutyl nitrite via inhalation for 24 months. Statistically significant increases in the incidence of lung alveolar/bronchiolar

carcinoma/adenoma in male and female rats and mice were observed. Cancer potency estimates based on the dose-response data in rats and mice are provided in Table 2. The potency estimate calculated based on the data in female mice was similar to that obtained based on the male rat data. Because of greater uncertainty in the interpretation of the dose-response data for female mice (control: 6/51; low dose: 15/51; mid-dose: 9/50; high dose: 19/50), the dataset in male rats was selected as the basis for the human cancer potency. The corresponding dose-response data are shown in Table 3. The human cancer potency for isobutyl nitrite is estimated to be 0.095 $(mg/kg\text{-}day)^{-1}$ and the associated NSRL is 7.4 μg/day.

Table 2: Values Used in Calculating Human Cancer Potency Values for Isobutyl Nitrite Based on the Incidence of Lung Alveolar/Bronchiolar Carcinoma/Adenoma (Combined) from NTP (1996).

Sex/species	q_1* $(mg/kg\text{-}day)^{-1}$	Interspecies Scaling Factor $(kg/kg)^{1/3}$	Correction for Experiment Duration $(wk/wk)^3$	q_{human} $(mg/kg\text{-}day)^{-1}$	Goodness-of-Fit Test[1]
Male rat	0.0183699	$(70/0.5)^{1/3}$	$(104/104)^3$	0.095	p = 0.4251
Female rat	0.00642053	$(70/0.35)^{1/3}$	$(104/104)^3$	0.038	p = 0.3712
Male mouse	0.00302404	$(70/0.03)^{1/3}$	$(104/104)^3$	0.040	p = 0.3714
Female mouse	0.00832995	$(70/0.025)^{1/3}$	$(104/104)^3$	0.12	NA[2]

[1] A p-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.
[2] Not applicable. To obtain an adequate fit, both the mid- and high-dose group data were dropped from the analysis, following Anderson *et al.* (1983).

Table 3: Incidence of Lung Tumors in Male F344 Rats Treated with Isobutyl Nitrite Via Inhalation (NTP, 1996).

Average Dose[1] (mg/kg-day)	Lung Alveolar/ Bronchiolar Carcinoma/ Adenoma Combined	Statistical Significance[2]
0	1/46	--
8.19	5/46	p = 0.1016
16.4	13/46	p < 0.001
32.8	15/46	p < 0.001

[1] As reported by CPDB.
[2] P-value listed next to dose groups is the result of pairwise comparison with the control group using the Fisher exact test.

NALIDIXIC ACID (CAS NO. 389-08-2)

Results from the studies by NTP (1989) and Kurokawa *et al.* (1986) were listed in the CPDB. Kurokawa *et al*. (1986) did not report any statistically significant oncogenic effects. NTP (1989) exposed male and female B6C3F$_1$ mice and male and female F344 rats to nalidixic acid via diet for 24 months. Statistically significant increases in the incidence of preputial gland tumors in male rats and clitoral gland tumors in female rats were observed. Cancer potency estimates based on these dose-response data are provided in Table 4. As indicated in Table 4, the most sensitive sex/species/site is male rat preputial gland. The corresponding dose-response data are shown in Table 5. The human cancer potency for nalidixic acid is estimated to be 0.025 (mg/kg-day)$^{-1}$ and the associated NSRL is 28 µg/day.

Table 4: Values Used in Calculating Human Cancer Potency Values for Nalidixic Acid Based on NTP (1989).

Sex/species/site	q_1* (mg/kg-day)$^{-1}$	Interspecies Scaling Factor (kg/kg)$^{1/3}$	Correction for Experiment Duration (wk/wk)^3	q_{human} (mg/kg-day)$^{-1}$	Goodness-of-Fit Test[1]
Male rat preputial gland (adenoma, papilloma, carcinoma combined)	0.0048229	$(70/0.5)^{1/3}$	$(104/104)^3$	0.025	p = 0.09994
Female rat clitoral gland (adenoma, papilloma, carcinoma combined)	0.00261296	$(70/0.35)^{1/3}$	$(104/104)^3$	0.015	p = 0.2729

[1] A p-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

Table 5: Incidence of Preputial Gland Tumors in Male F344 Rats Treated with Nalidixic Acid Via Diet (NTP, 1989).

Average Dose[1] (mg/kg-day)	Preputial Gland Carcinoma, Adenoma, Papilloma Combined	Statistical Significance[2]
0	3/50	--
79.2	19/50	p < 0.001
159	20/50	p < 0.001

[1] As reported by CPDB.

[2] P-value listed next to dose groups is the result of pairwise comparison with the control group using the Fisher exact test.

O-PHENYLENEDIAMINE (CAS NO. 95-54-5)

The human cancer potency estimate for *o*-phenylenediamine was calculated by applying a molecular weight correction (the ratio of the molecular weight of the dihydrochloride to that of the parent compound: 181.1/108.1) to the estimate for *o*-phenylenediamine dihydrochloride (discussed below). The resulting human cancer potency estimate is 0.027 $(mg/kg\text{-}day)^{-1}$, with an associated NSRL of 26 μg/day.

O-PHENYLENEDIAMINE DIHYDROCHLORIDE (CAS NO. 615-28-1)

Results from the studies by Weisburger *et al.* (1978) were listed in the CPDB. Male and female CD-1 HaM/ICR mice and male Charles River CD rats were exposed via diet for 18 months. Statistically significant increases in the incidence of liver tumors were observed in male rats and female mice. Cancer potency estimates based on these dose-response data are provided in Table 6. As indicated in Table 6, the most sensitive sex/species/site is male rat liver. The corresponding dose-response data are shown in Table 7. The human cancer potency for *o*-phenylenediamine dihydrochloride is estimated to be 0.016 $(mg/kg\text{-}day)^{-1}$ and the associated NSRL is 44 μg/day.

Table 6: Values Used in Calculating Human Cancer Potency Values for *o*-Phenylenediamine Dihydrochloride Based on Weisburger *et al.* (1978).

Sex/species/site	q_1* $(mg/kg\text{-}day)^{-1}$	Interspecies Scaling Factor $(kg/kg)^{1/3}$	Correction for Experiment Duration $(wk/wk)^3$	q_{human} $(mg/kg\text{-}day)^{-1}$	Goodness-of-Fit Test[1]
Male rat liver (benign and malignant combined)	0.0031189	$(70/0.5)^{1/3}$	$(104/104)^3$	0.016	p = 0.5104
Female mouse liver (benign and malignant combined)	0.0005790270	$(70/0.025)^{1/3}$	$(104/90)^3$	0.013	p = 0.2886

[1] A p-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

Table 7: Incidence of Liver Tumors in Male Charles River CD Rats Treated with *o*-Phenylenediamine Dihydrochloride Via Diet (Weisburger *et al.*, 1978).

Average Dose[1] (mg/kg-day)	Liver Tumors	Statistical Significance[2]
0	0/16	--
57.6	0/14	p = 1.00
115	5/16	p < 0.05

[1] As reported by CPDB.

[2] P-value listed next to dose groups is the result of pairwise comparison with the control group using the Fisher exact test.

REFERENCES

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group (1983). Quantitative approaches in use to assess cancer risk. *Risk Anal* **3**:277-295.

Crouch E (1992). MSTAGE (Version 1.1). E.A.C. Crouch, Cambridge Environmental Inc., 58 Buena Vista Road, Arlington, Massachusetts 02141.

Crump KS, Guess HA and Deal LL (1977). Confidence intervals and test of hypotheses concerning dose-response relations inferred from animal carcinogenicity data. *Biometrics* **33**:437-451.

Crump KS, Howe RB, Van Landingham C and Fuller WG (1993). TOX_RISK Version 3. TOXicology RISK Assessment Program. KS Crump Division, Clement International Corporation, 1201 Gaines Street, Ruston, Louisiana 71270.

Gold LS and Zeiger E (1997). *Handbook of Carcinogenic Potency and Genotoxicity Databases*. CRC Press, Inc. Boca Raton.

Gold LS, Manley NB, Slone TH and Rohrbach L (1999). Supplement to the Carcinogenic Potency Database (CPDB): Results of animal bioassays published in the general literature in 1993 to 1994 and by the National Toxicology Program in 1995 to 1996. *Environ Health Perspect* **107** (Suppl. 4):527-600.

Hoover SM, Zeise L, Pease WS, Lee LE, Hennig MP, Weiss LB and Cranor C (1995). Improving the regulation of carcinogens by expediting cancer potency estimation. *Risk Anal* **15**(2):267-80.

Kurokawa Y, Matsushima Y, Imazawa T, Takamura N, Maekawa A, Takahashi M and Hayashi Y (1986). Long-term *in vivo* carcinogenicity study of nalidixic acid in CDF1 mice. *Food Chem Toxicol* **24**:319-323.

National Toxicology Program (NTP, 1989). *Toxicology and Carcinogenesis Studies of Nalidixic Acid (CAS No. 389-08-2) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies).* NTP Technical Report Series No. 368. NIH Publication No. 90-2823. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

National Toxicology Program (NTP, 1996). *Toxicology and Carcinogenesis Studies of Isobutyl Nitrite(CAS No.542-56-3) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies).* NTP Technical Report Series No. 448. NIH Publication No. 96-3364. U.S. Department of Health and Human Services, NTP, Research Triangle Park, NC.

Office of Environmental Health Hazard Assessment (OEHHA, 1992). *Expedited Cancer Potency Values and Levels for Certain Proposition 65 Carcinogens.* California Environmental Protection Agency, OEHHA, Reproductive and Cancer Hazard Assessment Section, April 1992.

Weisburger EK, Russfield AB, Homburger F, Weisburger JH, Boger E, Van Dongen CG and Chu K (1978). Testing of twenty-one aromatic amines or derivatives for long-term toxicity or carcinogenicity. *J Environ Pathol Toxicol* **2**:325-356.