# NO SIGNIFICANT RISK LEVELS (NSRLs)
# FOR THE PROPOSITION 65 CARCINOGENS
# PHENYLHYDRAZINE AND PHENYLHYDRAZINE HYDROCHLORIDE

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## SUMMARY OF FINDINGS

The cancer potencies of phenylhydrazine and phenylhydrazine hydrochloride were estimated from dose-response data from three data sets: lung tumors among male and female mice (combined) exposed orally to phenylhydrazine hydrochloride (Clayson *et al.*, 1966) and blood vessel tumors among male and female mice exposed orally to phenylhydrazine hydrochloride (Toth and Shimizu, 1976). Studies of phenylhydrazine were of poor quality and were not used to derive potency estimates. 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 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. Cancer potency for phenylhydrazine hydrochloride was taken as the geometric mean of potencies derived from each data set analyzed. The cancer potency estimate obtained from studies of phenylhydrazine hydrochloride was used as the basis for the phenylhydrazine cancer potency by 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 Phenylhydrazine and Its Hydrochloride**

| Chemical | Cancer Potency $(mg/kg\text{-}day)^{-1}$ | NSRL ($\mu g/day$) |
|---|---|---|
| *Phenylhydrazine* | 0.68 | 1.0 |
| *Phenylhydrazine hydrochloride* | 0.51 | 1.4 |

## INTRODUCTION

This report describes the derivation of cancer potency values and no significant risk levels (NSRLs) for phenylhydrazine (CAS number 100-63-0, molecular weight 108.14) and phenylhydrazine hydrochloride (CAS number 59-88-1, molecular weight 144.60). "Phenylhydrazine and its salts" were listed on July 1, 1992 as known to the State to cause cancer under Proposition 65 (California Health and Safety Code 25249.5 *et seq*.). Phenylhydrazine and its salts, including phenylhydrazine hydrochloride, are not known to occur naturally but are widely used in the manufacture of dyes, nitron (a stabilizer for explosives), antipyrine, other pharmaceuticals and other chemicals; as reagents for the production of sugars, aldehydes, and ketones; and as reducing agents (HSDB, 2000).

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


**STUDIES SUITABLE FOR DOSE-RESPONSE ASSESSMENT**

The carcinogenicity of phenylhydrazine or phenylhydrazine hydrochloride was investigated in four series of studies (Clayson *et al.*, 1966; Roe *et al.*, 1967; Kelly *et al.*, 1969; Toth and Shimizu, 1976).  In these studies, the test compounds were administered orally to rodents.  Studies by Roe *et al.* (1967) and Kelly *et al.* (1969) were not as suitable for cancer potency estimation as those of Clayson *et al.* (1966) and Toth and Shimizu (1976), due to poor survival of the study animals and/or short study duration.

Clayson *et al*. (1966) administered phenylhydrazine hydrochloride by gavage, seven days per week for 42 weeks to BALB/c/Cb/Se mice of both sexes.  The total dose received over the 42 week dosing period was 200 mg.  An untreated mouse of equivalent age was sacrificed whenever a treated mouse died.  The experiment was terminated at 59 weeks.  An increased incidence of combined lung adenoma and carcinoma was observed in the treated group compared to controls.  These data are summarized in Table 2.  The authors observed 24 total lung tumors among 16 treated mice, and categorized each of the tumors into one of three groups based on the morphological degree of malignancy.  Of the 24 tumors in the treated group, 10 were classified as 'adenoma', 10 as 'adenoma in transition to malignancy', and 4 as 'malignant.'  Although the malignancy status of the lung tumors observed among the control animals was not reported, Clayson et al. (1966) noted that the degree of malignancy of the lung tumors observed in the phenylhydrazine hydrochloride-treated animals was greater than that observed in animals treated with either of four other agents tested concurrently.

**Table 2. Incidence of Lung Adenoma or Carcinoma (Combined) in BALB/c/Cb/Se Mice Treated with Phenylhydrazine Hydrochloride via Gavage (Clayson *et al*., 1966)**

| Administered Dose[1] (mg/day) | Lifetime Average Dose[2] (mg/kg-day) | Tumor Incidence | Statistical Significance[3] |
|---|---|---|---|
| 0 | 0 | 4/30 | -- |
| 1 | 17.6 | 16/30 | p = 0.001 |

[1] Treated and control groups, comprised of animals of both sexes (numbers of males and females not reported), were treated by oral gavage with 1 mg/day for 200 days in 42 weeks; the total administered dose was 200 mg.

[2] Lifetime average dose was calculated by dividing the total administered dose of 200 mg by the experimental lifetime of the mice in days (59 weeks x 7 days/week) and the average of the default body weights of female and male mice (0.0275 kg; Gold and Zeiger, 1997).

[3] Results of pairwise comparison using the Fisher Exact Test.

Toth and Shimizu (1976) exposed five- to six-week old male and female Swiss albino mice to 0.01% phenylhydrazine hydrochloride in drinking water for life. The incidence of combined blood vessel angiosarcoma and angioma was increased in treated male and female mice compared to the historical controls for the colony (Toth and Shimizu, 1974). The dose-response data are presented in Table 3.

**Table 3. Incidence of Blood Vessel Angiosarcoma and Angioma (Combined) in Swiss Albino Mice Treated with Phenylhydrazine Hydrochloride via Drinking Water (Toth and Shimizu, 1976)**

| Sex, Species | Administered Dose (% in drinking water) | Lifetime Average Dose[1] (mg/kg-day) | Tumor Incidence[2] | Statistical Significance[3] |
|---|---|---|---|---|
| Male mice | 0 | 0 | 6/99 | -- |
| | 0.01 | 27.0 | 10/49 | p <0.05 |
| Female mice | 0 | 0 | 5/99 | -- |
| | 0.01 | 25.2 | 11/49 | p <0.01 |

[1] Toth and Shimizu (1976) reported average chemical intakes of 0.81 mg/day for males and 0.63 mg/day for females. Lifetime average dose was calculated by dividing the intake by the default body weight (0.03 for male mice and 0.025 for female mice; Gold and Zeiger, 1997).

[2] Control tumor incidences for the colony were reported in Toth and Shimizu (1974).

[3] Results of pairwise comparison using the Fisher Exact Test.

**APPROACH TO DOSE RESPONSE ANALYSIS**

Phenylhydrazine and its salts were mutagenic in bacteria, with or without metabolic activation, and were genotoxic in mammalian cells *in vitro* (NIOSH, 1978; Brook, 1997). These findings are strongly suggestive that a genotoxic mode of action is plausible. There is insufficient information on the precise mechanism of carcinogenicity to permit the development of a biologically based model for cancer potency estimation. 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 approach used is described in detail in the Appendix.

## DOSE-RESPONSE ASSESSMENT

Cancer potency estimates were derived from the studies described above.  The cancer potency estimates are summarized in Table 4.  A cancer potency estimate of 4.3 $(mg/kg\text{-}d)^{-1}$ was obtained from the Clayson *et al.* (1966) study of BALB/c/Cb/Se mice, which included adjustments for the shortened study duration and rodent-human differences in body size.  From the Toth and Shimizu (1976) studies of Swiss albino mice, cancer potency estimates of 0.15 $(mg/kg\text{-}d)^{-1}$ for males and of 0.20 $(mg/kg\text{-}d)^{-1}$ for females were estimated.  Although the Clayson *et al.* study was of shorter duration (59 weeks) than the Toth and Shimizu (1976) studies (lifetime), Clayson *et al.* observed tumors at a different site (lung) than that of Toth and Shimizu (1976) (blood vessels).  The lung tumor findings of Clayson *et al.* (1966) appear consistent with other reports that the structurally related compound hydrazine sulfate induced lung tumors in BALB/c/Cb/Se mice (CancerChem, 2000).  Also, the incidence of lung tumors among control mice reported in Clayson *et al.* (1966) appears consistent with other studies published in the 1960s and 1970s using the same strain of mouse, as rates of lung tumors among control BALB/c/Cb/Se mice ranged from 0 % to 24% among males and 3 % to 18% among females (CancerChem, 2000).

**Table 4.  Human Cancer Potency Estimates for Phenylhydrazine and Phenylhydrazine Hydrochloride**

| Chemical | Cancer Potency Estimate $(mg/kg\text{-}day)^{-1}$ |
|---|:---:|
| **Phenylhydrazine Hydrochloride**: <br> Clayson *et al.* (1966) <br>     *BALB/c/Cb/Se mice* | <br><br>4.3 |
| Toth and Shimizu (1976) <br>     *Male Swiss albino mice* <br>     *Female Swiss albino mice* | <br>0.15 <br> 0.20 |
| *Overall Geometric Mean (3 studies)* | **0.51** |
| **Phenylhydrazine**[1] | **0.68** |

1    Cancer potency estimate for phenylhydrazine derived by multiplying the estimate for phenylhydrazine hydrochloride by the molecular weight of the hydrochloride divided by the molecular weight of phenylhydrazine (144.60/108.14).

Since both sets of studies were of similar quality and suitable for potency estimation, and since there is often lack of site concordance between rodent and human responses to carcinogens, the two potency estimates from the Toth and Shimizu studies were combined with the estimate from the Clayson *et al.* study.  The geometric mean of the results from the three studies yields a human cancer potency for phenylhydrazine hydrochloride of 0.51 $(mg/kg\text{-}day)^{-1}$.  Taking the geometric mean, as opposed to the arithmetic mean, provides some control for values that may be outliers that dominate the magnitude of the combined estimate.  To derive the human cancer

potency estimate for phenylhydrazine, a molecular weight conversion was applied to the potency for the hydrochloride (see footnote to Table 4).

## 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 (Table 4) were used to calculate NSRLs for phenylhydrazine (1.0 µg/day) and phenylhydrazine hydrochloride (1.4 µg/day).

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**APPENDIX: DEFAULT METHODOLOGY USED TO DERIVE NSRLS FOR PHENYLHYDRAZINE AND PHENYLHYDRAZINE HYDROCHLORIDE**

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 phenylhydrazine and phenylhydrazine hydrochloride 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, 1996; Anderson *et al.*, 1983):

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

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). 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:

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

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:

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

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 for each of the relevant dose groups, based on the dose level, duration and regimen described in the experiments above. When actual body weight information was not provided by the study authors, default values were utilized as described by Gold and Zeiger (1997). In this case, default body weights for male and female mice (30 and 25 grams, respectively) were taken from Gold and Zeiger (1997).

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

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

## A.3 Risk-Specific Intake Level Calculation

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

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

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:

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

**APPENDIX REFERENCES**

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