NO SIGNIFICANT RISK LEVEL (NSRL) FOR THE PROPOSITION 65 CARCINOGEN 2,4,6-TRINITROTOLUENE

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SUMMARY OF FINDINGS

The human cancer potency of 2,4,6-trinitrotoluene (TNT) was estimated and used to calculate a "No Significant Risk Level" (NSRL). The human cancer potency was estimated from dose-response data using the linearized multistage model for combined malignant lymphoma or leukemia of the spleen in female $B6C3F_1$ mice exposed via their feed (Furedi *et al.*, 1984b). The potency derivation takes into account differences in body size between humans and experimental animals. The human cancer potency estimate for TNT is 0.085 $(mg/kg\text{-}day)^{-1}$.

The Proposition 65 NSRL is defined in regulation as the daily intake level posing a 10^{-5} lifetime risk of cancer. The NSRL for TNT is calculated to be 8.2 μg/day.

Table 1. Cancer potency and NSRL for 2,4,6-Trinitrotoluene.

Chemical	Cancer Potency $(mg/kg\text{-}day)^{-1}$	NSRL (μg/day)
2,4,6-Trinitrotoluene	0.085	8.2

INTRODUCTION

This report describes the derivation of a human cancer potency estimate and NSRL for TNT (CAS No. 118-96-7). TNT was listed on December 19, 2008 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*.).

TNT is one of the most commonly used explosives for military and industrial applications. Commercially, TNT has been used in coal and mineral mining, deep-well and underwater blasting, and building demolitions. In chemistry it is used as an intermediate to generate charge transfer salts (Budavari, 1989). Other uses include use as a chemical intermediate in the manufacture of dyes and photographic materials (Lewis, 1997).

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

The available human data on cancer risk associated with TNT exposure consists of several case reports of liver cancer and leukemia in individuals exposed occupationally to TNT (e.g., Garfinkel *et al*., 1988; Yan *et al*., 2002), and three epidemiology studies that investigated the risk of cancer associated with TNT exposure: an ecologic epidemiology study (Kolb *et al*., 1993), a population-based case-control study (Kilian *et al*., 2001), and an historical occupational cohort study (Yan *et al*., 2002). None of these studies were of sufficient study design to provide the type of information necessary (e.g., individual TNT exposure estimates) to form the basis of a cancer potency estimate.

Long-term carcinogenicity studies of TNT have been conducted in Fischer 344 rats (Furedi *et al.*, 1984a) and B6C3F_1 mice (Furedi *et al.*, 1984b) of both sexes. In the studies in Fischer 344 rats, males and females (75 animals/sex/dose group) received TNT at 0, 0.4, 2, 10 or 50 mg/kg-day in their diet for up to 24 months (Furedi *et al*., 1984a). Ten rats per sex per dose group were sacrificed at six and twelve months, with surviving animals sacrificed at the end of the 24-month treatment.

Survival rates and mean survival duration did not differ among control and treated groups of either sex. Dose-related reductions in body weight (5-14% reductions in mid-dose animals, and 30-33% reductions in high-dose animals) and food intake were observed in both male and female rats.

In female rats, the incidence of benign and malignant neoplasms of the urinary bladder (transitional epithelia) was significantly increased in the high-dose group [$p < 0.0001$] and occurred with positive trend [$p < 0.0001$]. Bladder tumors are rare in untreated rats of this strain.

No treatment-related tumors were observed in male rats.

The dose-response data for the combined urinary bladder papilloma or carcinoma from the Furedi *et al.* (1984a) study in female rats are presented in Table 2.

Table 2. Incidence of urinary bladder tumors in female rats administered TNT via feed for two years (Furedi *et al*., 1984a).

Sex, Strain, Species	Tumor site and type	Average daily dose[a] (mg/kg-day)	Tumor incidence[a]	Statistical significance[b]
Female Fischer 344 Rats	Urinary Bladder Papilloma or Carcinoma	0	0/54	$p < 0.0001$[c]
		0.4	0/54	NS
		2.0	0/55	NS
		10.0	1/55	NS
		50.0	17/55	$p < 0.0001$

[a] As reported by Furedi *et al.* (1984a)
[b] Results of pairwise comparison using Fisher's Exact Test. NS is not significant.
[c] Cochran-Armitage trend test p-value.

In the studies of $B6C3F_1$ mice, males and females (75/sex/dose group) received TNT at 0, 1.5, 10, or 70 mg/kg-day in their diet for up to 24 months (Furedi *et al*., 1984b). Ten mice per sex per dose were sacrificed at six and twelve months, with surviving animals sacrificed after 24 months.

Survival rates were not altered among control and treated groups in either sex. A 10% reduction in body weight gain was observed in the first six to eight months of TNT administration in high-dose male and female mice, increasing to a 15% reduction in body weight gain in high-dose females, and a 20% reduction in body weight gain in high-dose males over the remainder of the treatment period.

In female mice, a positive dose-dependent increase in the incidence of malignant lymphoma and/or leukemia of the spleen was observed ($p < 0.05$). The incidence of these tumors was significantly elevated in high-dose females ($p < 0.01$) compared to controls. The observed leukemias were of the granulocytic or lymphatic type and the malignant lymphomas were histiocytic, lymphocytic, or mixed type.

No treatment-related tumors were observed in male mice.

The dose-response data for combined malignant lymphoma or leukemia of the spleen from the Furedi *et al.* (1984b) study in female mice are presented in Table 3.

Table 3. Incidence of malignant lymphoma/leukemia of the spleen in female mice administered 2,4,6-trinitrotoluene via feed for two years (Furedi *et al*., 1984b).

Sex, Strain, Species	Tumor site and type	Average daily dose[a] (mg/kg-day)	Tumor incidence[a]	Statistical significance[b]
Female $B6C3F_1$ Mice	Leukemia / Malignant Lymphoma of the Spleen	0	9/54	p = 0.0207 [c]
		1.5	15/54	NS
		10	17/54	p = 0.0571
		70	21/54	p = 0.0086

[a] As reported by Furedi *et al.* (1984b)
[b] Results of pairwise comparison using Fisher's Exact Test. NS is not significant.
[c] Exact trend test p-value.

APPROACH TO DOSE-RESPONSE ANALYSIS

This section briefly reviews the information on genotoxicity and other data relevant to possible mechanisms of TNT carcinogenicity for the purpose of determining the most appropriate approach for dose-response analysis.

OEHHA (2010) summarized the available information on the genotoxicity of TNT and its metabolites. Briefly, TNT has been shown to be genotoxic in many, but not all studies conducted in bacterial and mammalian systems *in vivo* and *in vitro*. It induced both frameshift and basepair substitution mutations in *Salmonella*, and mutations in mammalian cells *in vitro* in the Chinese hamster ovary cell hypoxanthine phosphoribosyl transfer (CHO-HPRT) locus assay and the mouse lymphoma thymidine kinase locus assay. TNT induced oxidative DNA damage in rat sperm *in vivo*, as measured by increased formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (reviewed in OEHHA, 2010).

Several metabolites of TNT have been assessed for genotoxic activity. For example, several TNT metabolites induced mutations in *Salmonella* [2-amino-dinitrotoluene; 4-amino-2,6-dinitrotoluene (4-ADNT); 2,6-diamino-4-nitrotoluene (2,6-DANT); 2,4-diamino-6-nitrotoluene] and CHO-HPRT assays (4-ADNT; 2,6-DANT). Another TNT metabolite, 4-hydroxylamino-2,6-dinitrotoluene, has been shown to damage DNA by increasing the formation of 8-oxodG and cleaving DNA at sites with consecutive guanines. In other studies, urine from workers exposed to TNT (containing urinary metabolites of TNT) is more mutagenic than urine from unexposed workers, when tested in the *Salmonella* assay (reviewed in OEHHA, 2010).

TNT binds covalently to proteins in humans (hemoglobin) and animals (hemoglobin, liver proteins), indicating the potential to bind to DNA (reviewed in OEHHA, 2010).

TNT can be metabolized through multiple pathways to form reactive nitroso species and reactive oxygen species, which may bind covalently with proteins and other

macromolecules, induce oxidative stress, and oxidative DNA damage (reviewed in OEHHA, 2010).

Structure activity comparisons with the carcinogenic nitrotoluenes 2,4-dinitrotoluene, 2,6-dinitrotoluene, and 2-nitrotoluene suggest that common pathways of metabolism and similarities in the reactivity of metabolic intermediates with proteins and DNA exist for TNT (reviewed in OEHHA, 2010).

While the mechanisms of carcinogenic action of TNT remain unclear, the evidence summarized above regarding its genotoxicity, metabolism to reactive species, and its similarity to other carcinogenic nitrotoluenes with respect to metabolism, reactivity of metabolic intermediates, and genotoxicity, suggests that TNT is likely to act through a genotoxic mechanism. Therefore, the default approach using a linearized multistage model is applied to derive a cancer potency estimate for each treatment-related tumor site. The default procedures are outlined in Title 27, California Code of Regulations, section 25703. A description of the methodology used is given in the Appendix.

DOSE-RESPONSE ASSESSMENT

Animal and human cancer potency estimates were derived for TNT by fitting the multistage model to the dose-response data from the Furedi *et al.* studies in female rats (1984a) and female mice (1984b) (Tables 2 and 3, respectively). The results are summarized in Table 4 below and the derivation of the estimates is described in the Appendix. Multiplying the animal cancer potency estimate derived from each experiment by the applicable interspecies scaling factor gives an estimate of human cancer potency.

The interspecies scaling factor is derived from the ratio of body weight in humans (assumed to be 70 kilograms [kg]) to the body weight of the experimental animals (as detailed in the Appendix). The average body weights of 0.245 kg for female rats and 0.0340 kg for female mice were calculated based on data reported by Furedi *et al.* (1984a, 1984b) for control animals.

As shown in Table 4, the human cancer potency derived from the female mouse study, 0.085 $(mg/kg\text{-}day)^{-1}$, is higher than that derived from the female rat study. The human cancer potency estimate for female mice is used as the basis for calculating the NSRL.

Table 4. Animal and human cancer potency estimates for 2,4,6-trinitrotoluene.

Sex, strain, species	Tumor Site and Type	Animal cancer potency $(mg/kg\text{-}day)^{-1}$	Human cancer potency $(mg/kg\text{-}day)^{-1}$
Female F344N rats	Urinary Bladder Papilloma or Carcinoma	0.00504	0.033
Female $B6C3F_1$ mice	Leukemia / Malignant Lymphoma of the Spleen	0.00669	**0.085**

Bold indicates the value selected as the basis for the NSRL.

NO SIGNIFICANT RISK LEVEL

The NSRL for Proposition 65 is the intake associated with a lifetime cancer risk of 10^{-5}. The human cancer potency estimate of 0.085 $(mg/kg\text{-}day)^{-1}$ for TNT, based on female mice, was used to calculate the NSRL for this chemical. The value of 8.2 μg/day was derived as shown below.

$$NSRL = \frac{10^{-5} \times 70\,kg}{0.085\,(mg/kg\text{-}day)^{-1}} \times 1000\,\mu g/mg = 8.2\,\mu g/day$$

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APPENDIX: METHODOLOGY USED TO DERIVE THE NSRL FOR 2,4,6-TRINITROTOLUENE

Procedures for the development of Proposition 65 NSRLs are described in regulation in Title 27, California Code of Regulations, sections 25701 and 25703. Consistent with these procedures, the specific methods used to derive the NSRL for 2,4,6-trinitrotoluene (TNT) are outlined in this Appendix.

A.1 Cancer Potency as Derived from Animal Data

Multistage polynomial model

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):

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

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 data. With four dose groups, as is the case with the Furedi *et al.* study (1984b) of TNT in female mice, the default linearized multistage model defaults to three stages, or four parameters, q_0, q_1, q_2, and q_3. The parameter q_0 provides the basis for estimating the background lifetime probability of the tumor (*i.e.*, $1 - \exp[-(q_0)]$. 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 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:

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

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:

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

Because the Furedi *et al.* (1984a, 1984b) studies of TNT were 104 weeks, a correction factor to extrapolate to 104 weeks was not required and therefore $q_{animal} = q_{1(UCB)}$.

Calculation of average daily dose

The Furedi *et al.* (1984a, 1984b) studies in rats and mice lasted 104 weeks and the feed was available to the test animals every day *'ad libitum'* except during 17-19 hour fasts (rats) or 2-5 hour fasts (mice) prior to blood collection or scheduled sacrifice. Individual body weight values were recorded weekly by Furedi *et al.* (1984a, 1984b) until test week 13 and biweekly until study termination. Food consumption was measured weekly for each cage of test animals until test week 13 and biweekly thereafter. Each animal's mean daily food consumption was calculated from these data. Weekly test diets for each treatment group, by sex, were prepared based on the body weight and food consumption data (Furedi *et al.;* 1984a, 1984b). Furedi *et al.* (1984a, 1984b) reported the following estimated average daily doses: for rats, 0.0, 0.4, 2.0, 10.0, and 50.0 mg/kg; for mice, 0.0, 1.5, 10.0, and 70.0 mg/kg.

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}) 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):

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

In the 1984 Furedi *et al*. studies, average body weights of 0.245 kg for female rats and 0.034 kg for female mice were calculated based on data reported for control animals; the default human body weight is 70 kg. An example derivation of human cancer potency using the female mouse animal cancer potency of 0.00669 $(mg/kg\text{-}day)^{-1}$ is shown below:

$$q_{human} = 0.00669\ (mg/kg\text{-}day)^{-1} \bullet (70\ kg\ /0.034\ kg\ )^{1/3} = 0.085\ (mg/kg\text{-}day)^{-1}$$

A.3 Risk-Specific Intake Level Calculation

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

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

where bw_h is the 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 27, California Code of Regulations, section 25703).

Thus for a 70 kg person, the NSRL is given by:

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

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