MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

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INTEROFFICE COMMUNICATION

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September 27, 1999

To: File for 2,4-Dinitrotoluene (CAS# 121-14-2)

From: Michael Depa, Toxics Unit, Air Quality Division

Subject: Screening Level Determination

The initial threshold screening level (ITSL) is 2 µg/m³ based on an 8-hour averaging time. The initial risk screening level (IRSL) for 2,4-dinitrotoluene(2,4-DNT) is 0.009 µg/m³ (annual average). The secondary risk screening level (SRSL) for 2,4-DNT is 0.09 µg/m³ (annual average).

The following references or databases were searched to identify data to determine the screening level: U.S. EPA Integrated Risk Information System (IRIS), Registry for Toxic Effects of Chemical Substances (RTECS), American Conference of Governmental and Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs), National Institute for Occupational Safety and Health (NIOSH) Pocket Guide to Hazardous Chemicals, Environmental Protection Bureau Library, International Agency for Research on Cancer (IARC) Monographs, Chemical Abstract Service (CAS) Online (1967- April 21, 1998), National Library of Medicine (NLM), Health Effects Assessment Summary Tables (HEAST), and National Toxicology Program (NTP) Status Report. The EPA has not established a reference concentration (RfC) for 2,4-DNT; however, the EPA established a reference dose (RfD) of 0.002 mg/kg/day. The ACGIH TLV for dinitrotoluene (mixed isomers) is 0.2 mg/m³. The NIOSH Recommended Exposure Level (REL) is 1.5 mg/m³. In 1996, IARC designated 2,4-DNT as “possibly carcinogenic to humans” (Group 2B). This was based on sufficient evidence in experimental animals and inadequate evidence in humans for the carcinogenicity of 2,4-DNT. The molecular weight of 2,4-DNT is 182.15g and the vapor pressure is 1.4×10^{-4} torr @ 25°C.

Overview of 2,4-DNT Toxicity

Acute toxic effects of 2,4-DNT exposures are caused by the ability of dintrotoluenes to produce methemoglobin (ACGIH, 1996) which decreased the oxygen-carrying capacity of the blood. Methemoglobinemia produces cyanosis accompanied by headache, irritability, dizziness, weakness, nausea, vomiting, dyspnea, drowsiness, unconsciousness, and possible death. Prolonged exposure can cause anemia. Excess heart disease (Levine et al. 1986) and hepatobiliary cancer mortality (Stayner et al. 1993) has been seen in workers exposed to 2,4-DNT.

Exposure to high levels of 2,4-DNT in animals regularly shows lower numbers of sperm and reduced fertility (ATSDR, 1998). Studies of animals have also shown a reduction in the numbers of red blood cells, nervous system disorders, and that liver and kidney damage can occur. Laboratory rats exposed to 2,4-DNT in the feed were found to have increased incidences of liver and skin cancer, and cancer of the mamary glands. In male mice, exposure to 2,4-DNT in the feed caused an increased incidence of kidney tumors. 2,4-DNT is considered a carcinogen pursuant to Rule 103(c) of the Air Pollution Control Rules promulgated under Article II, Chapter 1, Part 55 of the Natural Resources and Environmental Protections Act, 1994, PA 451.

Human Studies

Workers at two ammunition plants were investigated in an epidemiological study (Levine et al. 1986). Cohorts of 156 and 301 men who had worked a month or more during the 1940's and 1950's at jobs with opportunity for substantial technical grade dinitrotoluene (tDNT) exposure were followed through the end of 1980. The composition of tDNT was 75% 2,4-DNT, 19% 2,6-DNT and 5% other isomers. Co-exposure to nitroglycerine and ethylene glycol dinitrate was reported as minimal. No evidence of carcinogenic effect was found, but excesses of mortality from ischemic heart disease (IHD) were noted at both plants. The standardized mortality ratio (SMR) using mortality rates of U.S. men as the referent group for IHD at both plants was reported as 131 (95% confidence limit = 65-234) and 143 (95% CL = 107-187). When the local county mortality rates were used as the referent group the SMR for ischemic heart disease in the two plants was 133, and 138 (95% CL = 53-275 and 96-193, respectively). There was no information on smoking habits or other established risk factors for cardiovascular disease. However, the authors stress that smoking was prohibited in the plants and that there was no increase in mortality from lung cancer or respiratory diseases.

In an extended study of one of the ammunition factories studied by Levine et al. (1986), 4989 workers, who were employed for more than five months in jobs with probable exposure to a mixture of 98% 2,4-DNT and 2% 2,5-DNT, were followed until 1982 (Stayner et al. 1993). SMRs were estimated using U.S. mortality rates and standardized rate ratios (SRRs) were estimated using mortality rates from an internal unexposed cohort of 7436 workers at the same facility. An excess of cancer of the biliary passages, liver and gall-bladder (six cased) were observed in this study based upon comparisons with both the U.S. population (SMR 2.7; 95% confidence interval = 1.0-5.8) and the internal unexposed cohort (SRR 3.9; CI 1.0-14.4). No other cancer risks were observed. Using the same cohort, Stayner et al. (1992) found no increase in ischemic heart disease mortality.

Animal Studies

In a subacute and chronic study, groups of 6 male and female beagle dogs were dosed orally with 0, 0.2, 1.5, or 10 mg/kg 2,4-DNT for up to 24 months (Ellis et al., 1985). In the subacute study groups of 4 male and female dogs were given 0, 1, 5, or 25 mg/kg/day 2,4-DNT for 13 weeks. Blood samples were taken before treatment and at 3, 6, 9, 12 and 24 months. After 12 months, 1 pair from each group was killed for necropsy, and the treatment for another pair from each group was discontinued for 4 weeks to evaluate the reversibility. After 24 months, 2 pairs from each group were killed, and the treatment for 2 other pairs of each group was discontinued for 4 weeks, and these pairs were then necropsied. Extensive hematology tests were conducted. In addition to 9 organs, 27 tissues were examined for histopathology in each dog. The authors stated that the results in the subacute and chronic studies were qualitatively similar, with more pronounced effects of a more severe nature seen sooner in higher-dosed dogs. The main effects of 2,4-DNT toxicity were on the neural system and the erythrocytes; lesser effects were seen in the testes and the biliary tract. The neuropathology was characterized by incoordination and stiffness progressing towards paralysis. There were vacuolation, endothelial proliferation, and gliosis of the cerebellums of some affected dogs. These effects were seen in 1 dog given 1.5 mg/kg/day for 2 years, in all dogs given 10 mg/kg/day within 6 months, and in all dogs given 25 mg/kg/day within 2 months. The dogs given 25 mg/kg/day for 4 weeks and allowed to recover for 8 months had no CNS lesions. The authors stated that most dogs given 25 mg/kg/day in the subchronic study had mild to severe degeneration of the testes, with decreased spermatogenesis. This degeneration was not seen in any dogs given 10 mg/kg/day or less in the subchronic or chronic study. In the chronic, study hyperplasia of the biliary tract, including the gallbladder was observed in most dogs given 10 mg/kg/day and some dogs given 1.5 mg/kg/day (number of dogs presenting these lesions and the corresponding statistical values were not reported). After 3 months the male and female dogs dosed with 10 mg/kg had statistically significant decrease in the number of erythrocytes and % hemoglobin. The authors stated that neurotoxicity was most prominent in dogs and was the cause of early deaths and that it was only

a minor effect in rats and mice (see studies below). A LOAEL of 10 mg/kg was identified based on increased incidence of hyperplasia of the biliary tract, and decrease in the number of erythrocytes and % hemoglobin. A NOAEL of 1.5 mg/kg was also identified.

In a chronic study, groups of 25 male and female CD-1 mice were fed diets containing 0, 0.01, 0.07, or 0.5% 2,4-DNT (0, 14, 95, 898 mg/kg body weight per day) for up to 24 months (Hong et al., 1985). Extensive clinical and histopathology examination were performed. Toxic anemia was observed in high-dose male and female mice after feeding of 2,4-DNT for 12 months, and all mice in this dose group died by month 21. All high dose (898 mg/kg/day) males and females died by months 18 or 21, respectively. By the end of the study, the body weight of the low dose groups was comparable to the control mice. Erythrocytic effects seen in the high were those of toxic methemoglobinemia leading to Heinz bodies and concurrent anemia with reticulocytosis. The authors reported the incidence of biologically significant lesions (Table 1.).

Table 1. Incidence of Lesions in Mice fed 2,4-DNT for 24 months (Hong et al., 1985)

	Males				Females			
Dose (mg/kg)→	0	14	95	898	0	14	95	898
Renal (Kidney) Tumors	0/24	6/22*	16/19*	10/29*	0/23	0/20	0/22	1/23
Hepatocellular Dysplasia	1/25	17/25*	11/20*	31/32*	5/23	3/21	5/23	24/25*
Testicular or Ovarian Atrophy	8/24	3/25	13/20*	26/30*	1/29	2/23	0/27	15/24*

*P<0.05

In the Hong et al. (1985) study, renal tumors occurred at a statistically increased incidence (P<0.05) compared to controls in the male mice dosed with 95 and 898 mg/kg/day. The authors stated that, “The incidence of renal tumors in high males was less than that in middle-dose males, probably because some high-dose males had died before the tumor could develop.” The renal tumors consisted of cystic adenomas, cystic papillary adenomas, cystic papillary carcinomas and solid carcinomas, all of renal tubule origin. The authors stated that it was not uncommon to find more than one tumor type per mouse. The incidence of hepatocellular dysplasia was increased (P<0.05) in all dosed males and in the high dosed females compared to controls. The term “dysplasia” was used by the authors to “characterize metabolic, degenerative and hyperplastic alterations of cells.” Testicular atrophy and ovarian atrophy was increased (P<0.05) in the high dose males and females compared to controls. Clinical pathology findings in the dosed mice were similar to controls.

In another chronic feed study, groups of 38 male and female CD rats were fed diets of 2,4-DNT for 24 months (Lee et al, 1985). The dose of 2,4-DNT in the male rats was determined to be 0, 0.57, 3.92, or 34.5 mg/kg/day and the dose in the female rats was 0, 0.71, 5.14, 45.3 mg/kg/day. All high dose males and all but 1 high dosed female rats died by month 24. The feed intake was not affected during the 24-month study period; however, weight gains of both the high dose male and female rats were significantly reduced during most of the study. Statistically increased incidence of anemia (decreased erythrocyte count, hematocrit, and hemoglobin) was observed in the high dose male and female rats after 12 months of dosing. The authors stated that there was marked atrophy of the testes with severe atrophy of seminiferous tubules and almost complete lack of spermatogenesis in most high dose male rats fed 34 mg/kg. The incidence of selected lesions observed in rats during the chronic study are listed in Table 2.

Table 2. Incidence of Lesions in Rats Fed 2,4-DNT (Lee et al., 1985)

	Males				Females			
Dose (mg/kg)→	0	0.57	3.9	34	0	0.71	5.1	45

Hepatocellular Carcinoma	1/24	0/27	1/19	6/27	0/23	0/28	1/26	10/25*
Testicular atrophy	4/25	7/27	6/19	22/27*				
Mamary Gland Tumors					1/29	2/23	0/27	15/24*
Skin Tumors	2/25	4/27	3/19	15/27*	1/23	2/28	0/26	4/25
Pituitary adenoma	9/20	13/22	7/13	2/19	18/23	21/25	20/24	2/15

*P<0.05

In a bioassay, groups of 50 male and female rats and mice were fed doses of 2-4-DNT for 78 weeks and observed for an additional 26 weeks (NCI, 1978). The amount of 2,4-DNT fed to the rats was 0.008% for the low dose group (6.24 mg/kg/day for male rats and 7.36 mg/kg/day for the female rats) and 0.02% for the high dose group (15.6 mg/kg/day for the male rats and 18.4 mg/kg/day for the female rats). The amount of 2-4-DNT fed to the mice was 0.008% for the low dose group (13.68 mg/kg/day for the male mice) and 0.2% for the high dose group (68.4 mg/kg/day for the male mice and 69.6 mg/kg/day for the female mice). Each dose group had its respective control. At the end of the bioassay the high dose males weighed approximately 25% less than their controls. There was no significant weight depression in the low dose male rats. The same general weight depression was observed in the female rats. The approximate weight gain, expressed as a percentage of the weight gained by their respective control groups at the end of the bioassay, was 91% for low dose male mice, 82% for high dose male mice, 89% for low dose female mice, and 76% for high dose female mice. From this body weight data, it was concluded that the maximum tolerated dose was exceeded in the high dose male rats, both high and low dose male mice and high dose female mice. For male rats the Fisher exact test showed that the high-dose groups had a significantly (P=0.003) higher incidence of fibromas of the subcutaneous tissue and skin than the high-dose control (Table 3.). For the comparison of low-dose to low-dose control the Fisher exact test was also significant (P+0.008). In female rats the Fisher exact test indicated a significant (P=0.016) increase in fibroadenomas of the mammary gland in the high-dose compared to the high-dose control. For mice there were no tumors in either sex that had a statistically significant positive association between chemical administration and incidence. Non-neoplastic effect were mostly related to organs in which tumors had been found and were predominantly characterized by hyperplasia and metaplasia.

Table 3. Incidence of Lesions in Male and Female Rats (NCI, 1978)

	Low Dose Control	**High Dose Control**	**Low Dose**	**High Dose**
Male Rat Fibroma	0/46	0/25	7/49 (P=0.008)	13/49 (P=0.003)
Female Rat Mammary Gland Fibroadenoma	9/48	4/23	12/49	23/50 (P=0.016)

Discussion

The ATSDR (1997) reviewed the available toxicity information on 2,4- and 2,6-dinitrotoluene in 1997. The ATSDR stated that, "[B]ased on analysis of the urinary metabolites of workers in DNT manufacturing plants where inhalation exposure is considered the major route of exposure…it is apparent that absorption of these two compounds does occur." Since no information was found that indicated that the oral route to inhalation route extrapolation was inappropriate the oral toxicity data was deemed appropriate to use in the derivation of a screening level.

Derivation of Initial Threshold Screening Level (ITSL)

The EPA derived a RfD (reference dose) for 2,4-DNT at 0.002 mg/kg/day. According to Rule 232 (1)(b) the ITSL can be calculated as follows:

ITSL = RfD x 70kg/20m^3

ITSL = 0.002 mg/kg/day x 70kg/20m³

ITSL = 0.007 mg/m³

ITSL = 7 µg/m³ (24-hour averaging time)

An ITSL can also be developed from the ACGIH TLV of 0.2 mg/m³. The ITSL was calculated according to Rule 232(1)(c).

ITSL = OEL ÷ 100

Where the OEL is the occupational exposure limit. The ITSL then becomes

ITSL = 0.2 mg/m³ ÷ 100

ITSL = 0.002 mg/m³

ITSL = 2 µg/m³ (based on an 8-hour averaging time).

The TLV was of 2 µg/m³ was designed to be protective of acute methemoglobinemia (ACGIH, 1993) via the inhalation route, and the RfD was designed to be protective of neurologic effects via the oral route. The ITSL of 2 µg/m³ was chosen for the final ITSL in order to be protective of both hematologic and neurotoxic non-cancer health effects.

Derivation of Initial Risk Screening Level (IRSL)

The Global 82 program was used to derive oral slope factors for 2,4-dinitrotoluene from the available cancer bioassays. However, the chi-square statistic was found to be unacceptable in the Hong et al. (1985) study. Pursuant to Rule 231(3)(b) the highest dose was eliminated from the Hong et al. (1985) data used in the Global 82 program and the program was re-run. The raw slope factor data was then used in the animal to human extrapolation pursuant to Rule 231(3)(c) (see Table 4). The unit risk was calculated according to Rule 231(3(f)(ii). The final IRSL was derived from the Hong et al. (1985) study where an increased incidence of kidney tumors were observed in male mice.

Table 4. Derivation of Initial Risk Screening Level

Study	Tumor	Slope Factor	Unit Risk	IRSL
Hong et al (1985)	Γ Mice Kidney	0.02974 $(mg/kg)^{-1}$	0.00012 $(\mu g/m^3)^{-1}$	0.0087 µg/m³
Lee et al. (1985)	Γ Mice Skin	0.03264 $(mg/kg)^{-1}$	0.000047 $(\mu g/m^3)^{-1}$	0.021 µg/m³
Lee et al. (1985)	E Rat Liver	0.01650 $(mg/kg)^{-1}$	0.000027 $(\mu g/m^3)^{-1}$	0.037 µg/m³
Lee et al. (1985)	E Rat Mammary	0.01161 $(mg/kg)^{-1}$	0.000019 $(\mu g/m^3)^{-1}$	0.050 µg/m³
NCI (1978)	E Rat Mammary	0.0321 $(mg/kg)^{-1}$	0.000056 $(\mu g/m^3)^{-1}$	0.018 µg/m³
NCI (1978)	Γ Rat Skin	0.0301 $(mg/kg)^{-1}$	0.000048 $(\mu g/m^3)^{-1}$	0.021 µg/m³

Γ = male
E = female

In conclusion, the ITSL for 2,4-DNT is 2 µg/m³ with an 8-hour averaging time. The IRSL for 2,4-DNT is 0.009 µg/m³ with an annual averaging time. The secondary risk screening level (SRSL) for 2,4-DNT is 0.09 µg/m³ with an annual averaging time.

References

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