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HEALTH ADVISORY FOR HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX)

CRITERIA AND STANDARDS DIVISION
OFFICE OF DRINKING WATER
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

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PREFACE

This report was prepared in accordance with the Memorandum of Understanding between the Department of the Army, Deputy for Environment Safety and Occupational Health (OASA(I&L)), and the U.S. Environmental Protection Agency (EPA), Office of Drinking Water (ODW), Criteria and Standards Division, for the purpose of developing drinking water Health Advisories (HAs) for selected environmental contaminants, as requested by the Army.

Health Advisories provide specific advice on the levels of contaminants in drinking water at which adverse health effects would not be anticipated and which include a margin of safety so as to protect the most sensitive members of the population at risk. A Health Advisory provides health effects guidelines, and analytical methods and recommends treatment techniques on a case-by-case basis. These advisories are normally prepared for One-day, 10-day, Longer-term and Lifetime exposure periods where available toxicological data permit. These advisories do not condone the presence of contaminants in drinking water; nor are they legally enforceable standards. They are not issued as official regulations and they may or may not lead to the issuance of national standards or Maximum Contaminant Levels (MCLs).

The report is the product of the foregoing process. Available toxicological data, as provided by the Army, on the munitions chemical hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) have been reviewed and relevant findings are presented in this report in a manner so as to allow for an evaluation of the data without continued reference to the primary documents. This report has been submitted to critical internal and external review by the EPA.

A companion document, "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for RDX" is included in this report.

I would like to thank the authors (Dr. William L. McLellan, Dr. William R. Hartley and Dr. Margaret E. Brower) who provided the extensive technical skills required for the preparation of this report. I am grateful to the members of the EPA Tox-Review Panel who took time to review this report and to provide their invaluable input, and I would like to thank Dr. Edward Ohanian, Chief, Health Effects Branch, and Dr. Joseph Cotruvo, Director, Criteria and Standards Division, for providing me with the opportunity and encouragement to be a part of this project.

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EXECUTIVE SUMMARY

Hexahydro-1,3,5-trinitro-1,3,5-triazine, commonly known as RDX (British code name for Research Department Explosive or Royal Demolition Explosive), is a white crystalline solid that has been extensively used in military munitions formulations.

The pharmacokinetic properties of RDX have been extensively studied in rats. RDX was found to be completely absorbed via the oral route; the rate of absorption was reported to be a direct function of the particle size of the powder in the slurry administered. The rate of gastrointestinal absorption was found to be faster in rats than in humans or miniature swine; in rats, peak plasma levels were reached in 2 to 3 hours, whereas in swine and probably in humans, plasma levels peaked approximately 12 hours after dosing. Absorbed RDX is rapidly cleared from the plasma and distributed to tissues. The half-lives of clearance of RDX from plasma are of a similar order of magnitude in rats and humans: the $t_{1/2}$ was found to be 10.1 hours in rats and 15.1 hours in the one available human study. The highest RDX levels are found in the kidneys, followed by the levels in the liver, brain, and heart. RDX is metabolized by the liver, and its metabolites are excreted primarily in the urine. The metabolites have not been identified or characterized.

In humans, the toxic effects of RDX have been on the central nervous system (CNS). Exposure of workers in a munitions plant via inhalation of dust containing RDX has resulted in nausea, irritability, convulsions, unconsciousness, and amnesia. Military personnel have been exposed to RDX while burning composition C-4 explosives in the field to heat food; inhalation of the smoke resulted in clonic/tonic convulsions. Ingestion of RDX has caused similar CNS effects.

Acute toxicity studies indicated oral LD_{50} values of about 80 mg RDX/kg in mice and 118 mg RDX/kg in rats. Intravenous administration of single doses of RDX to beagle dogs caused convulsions and death at a dose of 40 mg/kg, central nervous system hyperactivity and nonlethal convulsions at a dose of 20 mg/kg, and decreased blood pressure and erratic electroencephalographic patterns at doses of 3.37 and 6.78 mg/kg.

Subchronic 90-day feeding studies in mice and rats indicate effects on the blood and liver. In mice of both sexes, increased liver weights were noted in groups receiving 320 mg RDX/kg/day, and anemia was seen in males receiving 160 mg RDX/kg/day. In rats, anemia was observed at a dose level of 28 mg RDX/kg/day in males, and increased liver weight was noted at a dose level of 100 mg RDX/kg/day in females. In a 10-day oral gavage study in monkeys, vomiting and convulsions were seen in five of six animals dosed with RDX at 10 mg/kg/day, but no central nervous system effects were observed at 1 mg/kg/day.

Lifetime feeding studies in rats and mice produced CNS effects, increased mortality, weight loss, anemia, hepatotoxicity, renal toxicity, testicular degeneration, and inflammation of the prostate. In male and female rats fed RDX in the diet at a level to give a daily intake of 40 mg/kg, tremors and convulsions, increased mortality, and enlargement of the liver were observed. Anemia and enlargement of the kidneys accompanied by histologic changes were also found in males receiving 40 mg RDX/kg/day. Inflammation of the prostate was found when RDX was administered at 1.5, 8, and 40 mg/kg/day; no effects were noted at a dose of 0.3 mg/kg/day. When mice were administered 175 mg RDX/kg/day, increased mortality was seen within 10 weeks. The high dose was reduced to 100 mg RDX/kg/day. Decreased weight gain was seen in females receiving 100 mg RDX/kg/day between 10 weeks postadministration and study

termination. Increased liver weights were found in males and females receiving RDX at 100 mg/kg/day, and testicular degeneration was found in males receiving 35 or 100 mg/kg/day; no important toxic effects were observed at 7 mg/kg/day.

RDX was not found to be mutagenic in bacteria and gave negative results in the dominant-lethal test and in an unscheduled DNA synthesis assay. RDX was not carcinogenic in rats. In $B6C3F_1$ mice, a significant increase was observed in the combined incidence of hepatocellular carcinomas and adenomas in females receiving RDX at 7, 35, or 100 mg/kg/day for 2 years. Mortality in mice receiving the highest dose was excessive, and the dose was lowered from 175 to 100 mg/kg at week 11. RDX is classified as Group C: Possible Human Carcinogen.

In a two-generation reproduction study in rats, decreased fertility was observed at 50 mg RDX/kg/day. Developmental effects (decreased pup weights) were seen at 16 and 50 mg RDX/kg/day; there were no effects at 5 mg/kg/day. RDX was found to be embryotoxic in rats at 20 mg/kg/day but was not found to be teratogenic. In a study in rabbits, RDX caused maternal toxicity at 20 mg/kg/day, and there was suggestive evidence for a teratogenic effect at 2 and 20 mg/kg/day.

Based on these findings and on the results of a 90-day oral toxicity study in monkeys where convulsions occurred in five of six animals administered 10 mg RDX/kg/day but no CNS effects were seen in monkeys administered 1 mg/kg/day, the Longer-term Health Advisory (HA) for a 10-kg child has been determined to be 0.1 mg/L (100 µg/L). In the absence of adequate animal data to determine a One-day or Ten-day Health Advisory, the Longer-term HA for a 10-kg child, 0.1 mg/L (100 µg/L), is used as a conservative estimate of the One-day or Ten-day HA. The Longer-term HA for an adult was established at 0.35 mg/L (400 µg/L). A Lifetime HA of 0.002 mg/L (2 µg/L) for an adult was determined based on a

Drinking Water Equivalent Level (DWEL) of 0.100 mg/L (100 µg/L). The DWEL is based on a Reference Dose (RfD) of 0.003 mg/kg/day where the effect was suppurative inflammation of the prostate of male rats fed RDX for 2 years.

I. INTRODUCTION

The Health Advisory (HA) Program, sponsored by the Office of Drinking Water (ODW), provides information on the health effects, analytical methodology, and treatment technology that would be useful in dealing with the contamination of drinking water. Health Advisories describe nonregulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations. Health Advisories contain a margin of safety to protect sensitive members of the population.

Health Advisories serve as informal technical guidance to assist Federal, State, and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. HAs are subject to change as new information becomes available.

HAs are developed for One-day, Ten-day, Longer-term (approximately 7 years, or 10% of an individual's lifetime), and Lifetime exposures based on data describing noncarcinogenic endpoints of toxicity. For those substances that are known or probable human carcinogens, according to the Agency classification scheme (Group A or B), Lifetime HAs are not recommended. The chemical concentration values for Group A or B carcinogens are correlated with carcinogenic risk estimates by employing a cancer potency (unit risk) value together with assumptions for lifetime exposure and the consumption of drinking water. The cancer unit risk is usually derived from the linear multistage model with 95% upper confidence limits. This provides a low-dose estimate of cancer risk to humans that is considered unlikely to pose a carcinogenic risk in excess of the stated values. Excess cancer risk estimates may also be calculated using the One-hit, Weibull, Logit, and Probit models. There is no

current understanding of the biological mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than another. Because each model is based upon differing assumptions, the estimates that are derived can differ by several orders of magnitude.

II. GENERAL INFORMATION

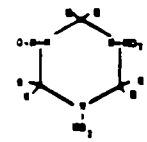
Hexahydro-1,3,5-trinitro-1,3,5-triazine (CAS No. 121-82-4), an explosive polynitramine, is commonly known as RDX (British code name for Research Department Explosive or Royal Demolition Explosive).

RDX, a white crystalline solid, is a completely N-nitrated, six-member heterocyclic ring compound. It has been extensively used as a high-impact explosive in military munitions formulations during and since World War II. RDX is also used as a rat poison (ACGIH, 1986; Windholz, 1983).

RDX is generally manufactured by the nitration of hexamethylene tetramine ($C_6H_{12}N_4$). In the United States, RDX is mainly manufactured at the Holston Army Ammunition Plant using the continuous Bachmann process (Pal and Ryon, 1986). This method involves the nitration of hexamine with ammonium nitrate and nitric acid in an acetic acid-acetic anhydride solvent (Sullivan et al., 1979).

General chemical and physical properties of RDX are presented in Table II-1. RDX (molecular weight 222.26) has approximately 130% the explosive power of trinitrotoluene (TNT) (Sullivan et al., 1979). It is considered a stable, relatively insensitive explosive, and can be stored up to 10 months at 85°C without perceptible deterioration (Meyer, 1977; Pal and Ryon, 1986). The low solubility of RDX in water (7.6 mg/L at 25°C and 1.3 g/L at 83°C) indicates that much of the compound detected in wastewater consists of undissolved particulates (Pal and Ryon, 1986). RDX preparations contain approximately 9% octahydro-1,3,5,7-tetranitro 1,3,5,7-tetrazocine (HMX).

Table II-1. General Chemical and Physical Properties of RDX

CAS No.	121-82-4
Synonyms	Cyclonite/Hexogen Cyclotrimetnylenetrinitramine Hexahydro-1,3,5-trinitro-1,3,5-triazine RDX sym-Trimethylenetrinitramine T_4 1,3,5-Trinitrohexahydro-s-triazine
Molecular weight	222.26
Empirical formula	$C_3H_6N_6O_6$
Chemical structure	
Physical state	White crystalline solid-orthorhombic crystal
Specific gravity	1.816 @ 20°C
Melting point	204.1°C
Heat of combustion	2,259.4 cal/g
Solubility characteristics:	
Water	0.00076% w/v @ 25°C (7.6 mg/L) to 42.3 mg/L (20°C) reported
Cyclohexanone	12.7% w/w @ 25°C
Cyclopentone	9.9% w/w @ 25°C
Acetone	8.3% w/w @ 25°C
Nitrobenzene	1.5% w/w @ 25°C
Methylisobutenyl ketone	3.0% w/w @ 25°C
Methylacetate	1.9% w/w @ 20°C
Acetic anhydride	4.9% w/v @ 30°C
Conversion factors (air)	1 ppm = 9.09 mg/m^3

SOURCE: Adapted from Hawley (1977); Small and Rosenblatt (1974); Windholz (1983); Sullivan et al. (1979); Etnier (1986).

III. SOURCES OF EXPOSURE

Occupational exposure may occur during manufacture and munitions incorporation of RDX. Human illness results from repeated exposure via the respiratory and gastrointestinal tracts and by skin absorption (Kaplan et al., 1965; ACGIH, 1986.) Reports of toxicity to humans were anecdotal and occurred in the early 1940s in Italy and Germany. All incidents were probably attributable to poor industrial hygiene procedures and inadequate ventilation, which resulted in contamination of workroom air with RDX dust. In one situation, no further cases of toxicity were observed when remedial protective measures were enforced (Kaplan et al., 1965). No air monitoring sampling was performed in the early studies. Hathaway and Buck (1977) surveyed U.S. munitions plants between 1972 and 1974 and found no adverse effects in workers with average 8-hour time-weighted exposures to RDX of 0.28 mg/m3 and maximum levels up to 1.57 mg/m3. Exposure to RDX in an occupational setting with standard hygiene and working procedures (e.g., protective clothing) is principally via airborne dust. In limited geographical areas, humans may be potentially exposed to RDX as an environmental contaminant.

RDX manufacturing operations such as recrystallization, dewatering, and incorporation are primary sources of RDX in wastewater. Load, assemble, and pack (LAP) facilities also discharge RDX-contaminated wastewater from such operations as washdown, explosive melting, and reject warhead steam cleaning. These wastewaters could contaminate groundwater and public drinking water supplies (Sullivan et al., 1979).

Wastewaters resulting from manufacture and loading of RDX may be discharged into the environment and may present a potential for aquatic pollution. Sediment

deposits in Army ammunition plants may also pose an environmental problem because seepage into the groundwater may occur (Etnier, 1986).

IV. ENVIRONMENTAL FATE

The environmental fate of RDX has been thoroughly reviewed by Etnier (1986). In experimental studies on migration of [^{14}C]RDX in soils of various pH, texture, and organic matter typically found in the United States, RDX was associated with downward movement and a very low leachate level (less than 0.5 ppm, which was the level of detection). Biological degradation to $^{14}CO_2$ near the surface of the soil was also observed. RDX is resistant to aerobic bacterial degradation in soil. However, in activated sludge systems, 97% of a 20-mg/L solution of RDX was degraded in 5 days. Bacterial degradation results in reduction to mono-, di-, and tri-nitroso derivatives, in reduction to a triazine, and in hydrolytic cleavage of the triazine. Formaldehyde, methanol, and CO_2 are end products. Photolysis rapidly degrades RDX. Volatilization is not significant in the environmental fate of RDX. Sediment absorption will not lead to a significant loss in the aquatic environment.

V. PHARMACOKINETICS

A. ABSORPTION

A dose, by gavage, of a slurry of coarse granular RDX did not cause convulsions in 10 Sprague-Dawley rats administered 100 mg/kg, whereas a gavage dose of a slurry of finely ground RDX caused convulsions in all 10 rats 2 hours after dosing at a level of 50 mg/kg. Measured particle sizes were not reported. The plasma concentration, 24 hours after dosing with the coarser RDX, was 3.04 µg/mL, whereas it was 4.7 µg/mL 24 hours after the smaller dose of finely ground RDX was administered. The LD_{50} value for the slurry of finely ground RDX was equivalent to that of a dimethylsulfoxide (DMSO) solution of RDX (Schneider et al., 1977). In preliminary studies with individual rats orally given a single 50-mg/kg dose of [^{14}C]RDX in DMSO, 83% of the administered radioactivity was found in the gut after 4 hours, 39.3% was found after 24 hours, and only 2.1% was found after 48 hours. Only 1 or 2% of the dose was found in the feces at 24 or 48 hours (Schneider et al., 1976).

Cholakis et al. (1980) dosed mice, by oral gavage, with much higher concentrations of RDX. Death occurred within 5 to 10 minutes at doses of 180, 225, and 350 mg/kg and within 30 minutes following a 140-mg/kg dose, indicating rapid absorption. From data of Schneider et al. (1977), it can be estimated that death would occur at plasma levels of about 14 µg/mL.

Absorption was slower in miniature swine than in rats. The peak level (4.7 µg/mL) was found in the plasma of swine 24 hours after a 100-mg/kg dose of finely ground RDX. Schneider et al. (1977) suggested that the rate of absorption via the gut in humans may be more comparable to the rate in swine, because the latent period preceding RDX convulsions in humans (Stone et al.,

1969) is of the same order as the period observed in swine and much longer than the period observed in rats.

B. DISTRIBUTION

Schneider et al. (1977, 1978) studied the tissue distribution, metabolism, and excretion of RDX in rats after single doses administered via parenteral and oral routes and after multiple oral doses. Ten Sprague-Dawley rats (both sexes) received a single intraperitoneal (ip) dose of 500 mg RDX/kg. Eight of the 10 rats died within 6 hours after experiencing severe clonic-tonic convulsions. The two rats that survived were sacrificed at 3.5 and 6.5 hours. The mean RDX plasma level (± SE) for all rats was 13.8±2.6 µg/g. The ratio of tissue concentration to plasma concentration was approximately 4.8 for kidney, 3.3 for liver, and 2.3 for heart or brain tissue. Two hours after an ip dose of 50 mg RDX/kg, the plasma level was 1.1 µg/g, and the tissue-to-plasma ratios for kidney, liver, heart, and brain tissue were 8.8, 5.7, 2.6, and 3.4, respectively. No convulsions were observed at the lower dose (Schneider et al., 1977).

After intravenous injection of 5 to 6 mg RDX/kg in 10 rats, there was a biphasic disappearance from the plasma. The half-life of the distribution phase was 6.32±0.18 minutes, the elimination phase had a half-life of 10.1±0.32 hours, and the apparent volume of distribution was 2.18 L/kg (Schneider et al., 1977). This high apparent volume of distribution may indicate significant binding to tissue.

The tissue distribution and RDX levels in the urine and plasma of rats at various times after an oral dose of 100 mg coarse granular RDX/kg are shown in Table V-1. Tissues were homogenized and extracted with benzene, and RDX was quantitated following gas chromatography with electron capture detection. The

Table V-1. Mean (± SE) RDX Concentration in Plasma, Urine, and Tissues of Rats at Various Times After Oral Administration of 100 mg RDX/kg[a]

Time (hr)	Plasma (µg/g)[b]	Urine (µg/mL)	Brain (µg/g)	Heart (µg/g)	Liver (µg/g)	Kidney (µg/g)
2	1.50±0.26	2.45±0.30	10.36±1.24	7.97±1.11	4.34±0.90	12.86±1.4
4	2.09±0.09	5.46±0.96	7.71±0.98	6.49±0.96	2.16±0.56	12.30±1.-
6	1.78±0.15	5.02±0.81	7.51±0.57	7.13±0.71	0.51±0.34	13.58±1.-
8	2.36±0.22	7.31±0.85	5.57±0.67	3.82±0.52	0.15±0.15	10.90±0.-
12	2.26±0.16	5.49±1.03	11.28±1.60	11.08±1.82	8.51±2.24	22.02±2.0
18	2.03±0.10	5.58±0.28	6.30±0.20	5.58±2.24	0.48±0.20	12.12±0.-
24	3.04±0.48	6.87±0.84	8.91±1.07	7.89±0.83	2.56±1.15	16.85±0.-

[a]Rats were fasted 24 hours prior to dosing.
[b]Conversion factor (micrograms per milliliter to micrograms per gram wet weight) = 0.9737.

SOURCE: Adapted from Schneider et al. (1977).

levels in kidney tissue were highest, followed by those in brain, heart, and liver tissue.

Tissue levels after oral dosing of rats with 50 mg/kg finely powered [^{14}C]RDX were also studied (Schneider et al., 1977). A time course of tissue concentrations was not presented. Concentrations of tissue radioactivity 24 hours after a gavage dose of 50 mg [^{14}C]RDX (finely powered material) were compared with a study using 100 mg of coarse granular RDX. It was assumed that all radioactivity was associated with RDX, and quantitation was accomplished by dividing the amount of radioactivity of tissue by the specific radioactivity of the administered RDX. No correspondence was observed between the 24-hour tissue levels in this experiment and those determined after gas-chromatographic quantitation of the 100-mg/kg nonradioactive dose. Since it has been shown that [^{14}C]RDX is metabolized to $^{14}CO_2$, it is conceivable that labeled activated one-carbon fragments are biosynthetically converted to tissue components (Schneider et al., 1977), thereby causing the apparent discrepancy.

Ten female miniature swine (21 to 60 kg) were fasted for a day and then administered 100 mg RDX/kg by oral gavage; four convulsed and two died at 22 hours. Plasma RDX concentrations reached about 1.8 µg/mL 1 hour after dosing; the level (measured at 30-minute intervals) remained fairly constant over 6 hours, then increased to 4.7 µg/mL prior to sacrifice at 24 hours postdosing. Tissue concentrations at 24 hours are shown in Table V-2. No marked accumulation of RDX was found in any tissue, except stomach and colon, when compared to plasma levels. This result is in contrast to that found for rats (Schneider et al., 1977). A time course of tissue radioactivity was not available for swine.

Table V-2. Mean (± SE) RDX Concentration in Plasma, Urine, Feces, and Tissues of Miniature Swine 24 Hours After Oral Administration of 100 mg RDX/kg

Tissue	RDX concentration[a]
Brain	7.0±1.9 (10)[b]
Heart	5.3±1.4 (10)
Liver	4.4±1.7 (10)
Renal cortex	9.1±4.3 (10)
Renal medulla	5.6±1.8 (10)
Spinal colon	18.7±11.0 (6)
Stomach	42.5±14.4 (6)
Fat	8.7±2.6 (9)
Feces	2.5±0.6 (6)
Plasma	4.7±2.1 (8)
Urine	3.6±1.1 (9)

[a]Tissue RDX concentrations are expressed in micrograms per gram of wet weight. Urine and plasma RDX are expressed in micrograms per milliliter.
[b]The number of animals sampled is in parentheses.

SOURCE: Adapted from Schneider et al. (1977).

In a 90-day study conducted by Schneider et al. (1978), groups of 18 Sprague-Dawley rats (both sexes) were provided with drinking water spiked with [^{14}C] RDX (50 to 70 µg/mL containing 7 to 18 µCi/mL). Based on measured water consumption, the daily intake of RDX was between 5 and 8 mg/kg. Groups of six rats were sacrificed at 30, 60, and 90 days to determine tissue distribution and body burden of RDX. Data for tissue levels are summarized in Table V-3. No accumulation of RDX was found in any of the tissues examined after 90 days, the levels in fat and brain tended to be slightly higher than those in other tissues. No overt toxic signs were reported.

In another 90-day study, 30 rats were administered, by gavage, 20 mg RDX/kg/day. Tissues were collected from groups of 10 animals sacrificed at 30, 60, and 90 days postdosing. In the brain, heart, liver, and kidney tissues, the RDX levels increased only slightly from 30 to 90 days. The mean tissue levels at 90 days (estimated from a bar graph) were approximately 10 µg/g for brain, heart, liver, and kidneys. The RDX level in fat was 7 to 8 µg/g dry weight at 30 and 60 days, and increased to approximately 20 µg/g dry weight at 90 days (Schneider et al., 1978).

C. METABOLISM AND EXCRETION

In a 90-day study, groups of six Sprague-Dawley rats (both sexes) were provided with drinking water spiked with RDX (50 to 70 µg/mL containing 7 to 8 µCi/mL [^{14}C]RDX). At weeks 1, 4, 8, and 13, radioactivity in exhaled $^{14}CO_2$, as well as in urine and feces, was determined. The results expressed as percent of administered daily dose are presented in Table V-4. From 27 to 50% of a daily dose was metabolized to $^{14}CO_2$ and exhaled, 22 to 35% was excreted in the urine, and 4 to 5% was excreted in the feces. The nature of the urinary and fecal radioactivity was not investigated. Some variability in recovery, which ranged from 54 to 89% (Schneider et al., 1978), was observed.

Table V-3. RDX Concentration of Tissues From Rats Provided With Drinking water Spiked with [^{14}C] RDX (50 to 70 µg RDX/mL) at 30, 60, and 90 Days

	RDX concentrations (µg/g)		
	30 days	60 days	90 days
Brain	0.59±0.31[a]	0.40±0.23	0.65±0.15
Heart	0.84±0.67	0.20±0.09	0.45±0.07
Liver	0.80±0.64	0.09±0.04	0.20±0.05
Kidney	0.30±0.14	0.12±0.04	0.47±0.11
Stomach	0.13±0.06	0.11±0.05	0.35±0.08
Colon	0.27±0.14	0.11±0.04	0.40±0.10
Fat	ND[b]	ND	0.57±0.04

[a]The values are mean ± SE for six rats for each determination.
[b]ND = Not determined.

SOURCE: Adapted from Schneider et al. (1978).

Table V-4. Daily Recovery of Radioactivity at Specific Intervals During Administration of Drinking Water Spiked With [^{14}C]RDX to Rats

Week of study	Percent of administered daily dose[a]			Total recovery (%)
	Exhaled CO_2	Urine	Feces	
1	35.4±2.7	35.3±2.5	4.0±0.8	74.7
4	34.8±2.0	24.6±1.6	4.0±0.3	63.4
8	50.5±3.9	33.7±1.3	5.3±0.8	89.4
13	27.4±0.8	22.2±1.5	4.3±0.8	53.9

[a] Mean ± SE.

SOURCE: Adapted from Schneider et al. (1978).

A 7-day study was carried out in five rats administered, by gavage, 20 mg/kg [^{14}C]RDX dissolved in DMSO. The percent of radioactivity recovered in exhaled CO_2, urine, and feces was 38.9±3.9, 33.6±2.3, and 4.8±0.8, respectively; total recovery was 77.3% of the daily administered radioactivity (Schneider et al., 1978).

The urinary metabolites of RDX have not been identified. However, less than 3% of a single dose of 50 mg/kg is excreted in the urine unchanged (Schneider et al., 1977). The residual RDX remaining in the carcass 4 days after a single dose was 0.30±0.05 µg/g, which corresponds to 0.6% of the original dose. However, when radioactivity was used to monitor residues in the carcass after 4 days, 9.5±0.03% of the radioactivity was found. This would suggest that metabolites such as bicarbonate and formaldehyde or formate (one-carbon fragments) may be biosynthetically incorporated into the tissues of the rats.

One study was found in which rates of excretion were determined in humans (Woody et al., 1986). A 3-year-old child weighing 14.5 kg ingested pellets of composition C-4 (91% RDX). The peak RDX concentration in serum was 10.74 mg/L at 24 hours and dropped to 3.56 and 0.66 mg/L at 48 and 96 hours, respectively. Disappearance of RDX from serum followed a monophasic exponential pattern, with a half-life of 15.06 hours. The concentration in the cerebrospinal fluid (CSF) was 8.94 mg/L at 24 hours, giving a ratio of 0.832 for CSF:serum. The rate of fecal excretion was slower than the rate of urinary excretion; peak fecal concentrations (4.49 mg/g) were reached at 96 hours compared with 48 hours for peak urine concentrations (38.4 mg/L). Using an assumed value of 2.2 L/kg for volume of distribution, it was estimated that a total of 1.23 g had been ingested (84 mg/kg).

VI. HEALTH EFFECTS

A. HUMANS

Chronic RDX exposure via the inhalation route has been characterized in munitions workers by epileptiform seizures (convulsions) and unconsciousness (Ryon et al., 1984). These convulsions may be preceded by insomnia, restlessness, and irritability, and they may be followed by temporary amnesia and disorientation. Convulsions have been reported primarily from exposure to the powdered form of RDX; complete recovery has been found to occur upon removal from exposure.

Kaplan et al. (1965) reported five case histories involving convulsions or unconsciousness among 26 employees in an explosives plant that was processing RDX. These cases occurred within 3 months of startup of RDX-processing operations. There were few prewarning symptoms; some workers had short periods of headache, dizziness, nausea, or vomiting. Two convulsed and became unconscious while working. One to several hours after leaving work, three others became unconscious without experiencing convulsions. These individuals were hospitalized and treated, and they generally recovered within 1 day. No abnormalities were observed in blood counts or urinalyses, and no symptoms other than CNS effects were noted. Workers were exposed to finely powdered RDX dust, and absorption was probably primarily via inhalation. When hygienic measures were taken in the plant, no further adverse effects were seen. Kaplan et al. (1965) cited previously reported cases involving industrial exposure to RDX in Russia, Italy, and Germany that resulted in epileptiform symptoms and unconsciousness.

Ketel and Hughes (1972) reported 40 cases of RDX intoxication in U.S. soldiers in Vietnam. In these cases, exposure was primarily via inhalation since

the soldiers frequently burned composition C-4 explosives to heat food in the field. Composition C-4 contained 91% RDX, 2.1% polyisobutylene, 2.1% motor oil, and 5.3% sebacate (a plasticizer). The patients showed symptoms of CNS toxicity ranging from confusion to multiple seizures followed by amnesia. Most patients (88%) experienced frequent nausea and vomiting. Three patients had one generalized clonic/tonic convulsion, and 44% had intermittent generalized convulsions that occurred from 24 to 36 hours after hospitalization. Phenytoin did not control the convulsions, but phenobarbital controlled myoclonic activity. Electroencephalograms (EEGs) were performed on 18 patients. EEGs performed at the time of convulsions showed bilateral synchronous spike and wave complexes (2 to 3/second) on the frontal areas; there was also diffuse slow wave activity. Eight days later, spike and wave complexes were absent, but the slow frequency background rhythm persisted. EEGs usually normalized within 1 to 3 months.

Hollander and Colbach (1969) also reported five cases of convulsions in which soldiers burning composition C-4 to heat food in the field were exposed to RDX or its combustion products.

Systemic intoxication was not found at a World War II munitions plant that employed a closed system of production. However, primary irritation and sensitization dermatitis of the face and eyelids occurred in workers exposed to production-related fumes. Patch testing with solid RDX failed to produce local skin reactions; the irritation and dermatitis experienced were determined to be related to an unidentified component in the fumes (Sunderman, 1944, as cited in Ryon et al., 1984).

Hathaway and Buck (1977) conducted a study of 93 munitions plant workers exposed to RDX and HMX; 69 workers were exposed to primarily RDX. The workers were evaluated for abnormalities of the hematologic, hepatic, and renal systems,

and for the presence of autoimmune disease. Atmospheric sampling of RDX was conducted intermittently for several hours over a 6-week period; sampling of HMX was not performed. Exposures ranged from undetectable to 1.57 mg RDX/m^3, with a mean detectable exposure of 0.28 mg RDX/m^3 . Duration of occupational exposures was not reported. Results of the fluorescent antinuclear antibody test, which had previously been used to demonstrate evidence of autoimmune disease (systemic lupus erythematosus or SLE), were found to be higher in the nonexposed controls (3.5%) than in the RDX-exposed employees (2.2%). Results of other blood chemistry and hematological tests were found to be similar between controls and RDX-exposed workers. The authors concluded that medical testing of RDX-exposed workers failed to reveal evidence of adverse health effects for exposures up to 1.57 mg RDX/m^3. Three cases of SLE previously found at one ammunition plant were reported to be unrelated to RDX exposure.

Stone et al. (1969) presented case reports on four military personnel who ingested from 25 to 180 g of C-4 plastic explosive (91% RDX). All were admitted to the hospital because of generalized convulsions. Between convulsions, the state of consciousness of the patients varied from coma to lethargy. Symptoms included muscular twitching, hyperactive reflexes, headaches, nausea, vomiting, hematuria, low-grade fever, and loss of memory. Although serum glutamic-oxaloacetic transaminase was elevated, there were no abnormal findings in liver function tests, and liver biopsies appeared normal. Kidney biopsies did not reveal any permanent histologic change. Recovery of mental capacity was complete after 1 to 2 months. No fatalities resulted.

Knepshield and Stone (1972) reported six cases of RDX intoxication in U.S. soldiers in Vietnam who ingested unreported concentrations of composition C-4. All patients exhibited symptoms of CNS toxicity including convulsions (100%),

coma (67%), disorientation and confusion. Mental capacities were reported to normalize within one to two months. Neuromuscular irritability (muscular twitching), gastrointestinal symptoms, hematuria and fever abated within forty-eight hours of ingestion. Bifrontal headaches, occurring in 50% of the patients, persisted for one to three weeks. The ingestion of larger concentrations of RDX were reported to produce extended periods of mental confusion and anemia. Similarly, Merrill (1968) reported case studies on two military personnel who ingested unreported concentrations of composition C-4. Symptoms of CNS toxicity (hyperirritability, muscle twitching, convulsions, mental confusion and amnesia), renal damage (oliguria, hematuria, elevated BUN) and hepatic involvement normalized within 10 days of ingestion.

Woody et al. (1985, 1986) reported a case of a 3-year-old, 14.7-kg child who was hospitalized in status epilepticus. It was determined that the child had ingested pellets of plasticized RDX explosive. The child's mother worked in a munitions plant, and clumps of the explosive had adhered to her boots and clothing. Blood, urine, and stool samples were taken serially, and samples of cerebrospinal fluid (CSF) were analyzed. CAT scans of the head were normal. An EEG revealed no evidence of epileptiform activity; however, it showed diffuse wave slowing, predominantly in the occipital regions. Hematology parameters and urinary function were normal. Serum glutamic-oxaloacetic transaminase increased from an initial value of 46 IU to 60 IU at 24 hours. Other serum chemistry parameters were normal. The CSF showed slight increases in protein and glucose compared with normal values. The level of RDX in the CSF at 24 hours, measured by high pressure liquid chromatography (HPLC), was 8.94 mg/L compared with 10.74 mg/L for serum. It was estimated that the amount ingested was 1.23 g (84.82 mg/kg). See Chapter V, Pharmacokinetics, for discussion of dose estimation.

B. ANIMAL EXPERIMENTS

1. Short-term Exposure

a. Acute

The acute oral LD_{50} values for RDX in mice and rats range from 59 to 97 mg/kg and from 71 to 300 mg/kg, respectively (Table VI-1). Schneider et al. (1977) reported that the acute oral LD_{50} value for RDX depends on its physical form and on the methods used to suspend or dissolve RDX. A single oral administration of 100 mg RDX/kg in a coarse granular preparation did not induce convulsions in Sprague-Dawley rats. However, when 10 rats received, by gavage, a 50-mg/kg dose of finely powdered RDX in a stable saline slurry, all rats experienced convulsions and 2 died. The LD_{50} value of either the finely powdered RDX in a saline slurry or DMSO solution was 100 mg/kg, whereas the LD_{50} value of the coarse granular RDX was approximately 300 mg/kg.

Cholakis et al. (1980) conducted single-dose oral toxicity studies in male and female $B6C3F_1$ mice and Fischer 344 rats. RDX was suspended in a 1% methylcellulose and 1% Tween-80 mixture in water and administered, by gavage, to groups of five male and five female mice at doses of 60, 100, 140, 180, 225, or 350 mg/kg body weight (bw). Groups of 10 male and 10 female rats received oral doses of 100, 125, 150, 180, 200, or 250 mg/kg bw of RDX suspended in 1% methylcellulose in water. The dosing volume was 20 mL/kg, and all surviving animals were observed daily for 14 days. The RDX used in the study was contaminated with 9% HMX.

In mice, death occurred within 5 to 10 minutes postdosing at the 180-, 225-, and 350-mg/kg levels. Within 30 minutes, 60 and 90% mortality occurred at the 100- and 140-mg/kg dose levels, respectively. At the lowest dose (60 mg/kg), three mice died on day 10 of the study. These animals exhibited central nervous

Table VI-2. Acute LD_{50} Values for RDX in Laboratory Animals

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg)	Reference
Mouse	Swiss-Webster	M	Oral	Corn oil	<75	Dilley et al. (1978)
		F			86 (8-124)[a]	
Mouse	$B6C3F_1$	M	Oral	Methyl-cellulose Tween-80	97.2±8.7 (81.6-115.8)	Cholakis et al. (1980)
		F			58.9±26.8 (24.8-139.5)	
		Combined			80.3±9.6 (55.3-99.2)	
Mouse	--[b]	--	Intra-venous	DMSO	18.71 (15.66-22.24)	McNamara et al. (1974)
Rat	Sprague-Dawley	M	Oral	Corn oil	71 (56-85)	Dilley et al. (1978)
		F			71 (50-75)	
Rat	Sprague-Dawley	M/F[c]	Oral	DMSO or saline slurry	100	Schneider et al. (1977)
Rat	Sprague-Dawley	M/F	Oral	Coarse powder	300	
Rat	Fischer 344	M	Oral	Methyl-cellulose	119.0±4.6 (110.4-128.3)[a]	Cholakis et al. (1980)
		F			8.7±4.5 (108.0-128.9)	
		Combined			118.1±2.8 (111.8-124.1)	
Rat	--	--	Oral	Gum acacia	200*	Von Oettingen et al. (1949)
Guinea pig	--	--	Intra-venous	DMSO	25.1 (20.0-31.6)	McNamara et al. (1974)

[a]95% confidence limits.
[b]Data not reported.
[c]Estimated LD_{50} for male or female.

*Approximate minimum lethal dose.

system signs that included gasping/labored breathing, Straub tail-like syndromes, and clonic/tonic convulsions prior to death. In rats, all the animals receiving the 150-, 180-, and 250-mg/kg doses, and 95% of those receiving the 200-mg/kg dose, died within 2 to 3 hours postadministration; the mortality rate was 70 and 5% for rats at the 125- and 100-mg/kg levels, respectively. Rats also exhibited central nervous system effects such as gasping/labored breathing and convulsions. In both species, there were no apparent sex differences in response to RDX administration, and gross necropsy did not reveal treatment-related changes in any tissues.

Von Oettingen et al. (1949) studied the acute toxicity of RDX in rats (strain and sex not specified). RDX was administered as a 1 to 4% suspension in gum acacia to groups of 5 to 15 rats. Doses delivered ranged from 25 to 400 mg RDX/kg. The authors established an approximate minimum lethal dose (MLD_{50}) of 200 mg/kg for RDX.

French et al. (1978) observed ultrastructural changes in the liver and kidneys of rats following a single oral dose of 100 mg RDX/kg. The liver and kidneys were examined 24, 48, and 120 hours postdosing. Examination at 24 hours revealed hepatocytes with dilation of the rough endoplasmic reticulum, mitochondrial swelling, and the presence of concentric membrane arrays. In the kidneys, changes were confined to the distal convoluted tubular cells; no consistent alterations were observed in the glomerular cells, proximal convoluted tubular cells, or the collecting tubular cells. At 48 hours, the liver showed proliferation of the smooth endoplasmic reticulum (SER); hepatocyte alterations were similar and of the same magnitude. No consistent changes were observed in the kidneys. At 120 hours, the hepatocyte alterations persisted and SER proliferation increased; no consistent changes were observed in the kidneys. The minimal and transient effect of RDX in the kidneys, and the persistence of

hepatocyte alterations and proliferation of the SER, indicate a possible induction of the mixed function oxidase (MFO) system. However, no data on MFO levels were presented.

In a study with dogs (breed and sex not reported), oral administration by stomach tube of 5 or 15 mg of RDX/kg caused little, if any, changes in the animals' blood pressure, respiratory rate, minute volume, and spinal fluid pressure (Von Oettingen et al., 1949).

McNamara et al. (1974) investigated the toxicity of RDX after intravenous (iv) dosing in mice, guinea pigs (strain and sex not specified), and beagle dogs. LD_{50} values are presented in Table VI-1.

Beagles were injected intravenously with solutions containing 33% RDX in DMSO, 7.5% in cyclohexanone, and 5.4% in acetone (w/v) in single doses of 0.125 mL/kg. Two dogs dosed at 40 mg RDX/kg in DMSO exhibited subconvulsive jerking, twitching, and convulsions 15 to 30 minutes after dosing; both dogs died within 90 minutes. The convulsions were cyclic, and dogs exhibited inadequate respiratory movements, decreased blood pressure (BP), and a flat line on the EEG. When administered a dose of 20 mg/kg, one dog exhibited CNS hyperactivity within 15 seconds after dosing and hyperreflexia for 1 hour; the other dog convulsed within 90 seconds and did not recover until 16 hours later. Dogs given 4.7 or 9.4 mg RDX/kg in cyclohexanone showed marked decrease in BP, cardiac arrest, respiratory inhibition, and low-frequency EEG discharge. Dogs were semicomatose to comatose, eyes were dilated, and the pain threshhold was elevated. Dogs given 3.37 or 6.78 mg RDX/kg in acetone showed decreased BP and erratic EEG disturbances (McNamara et al., 1974). The Lowest Observed Adverse Effect Level (LOAEL) for this study was 3.37 mg/kg; a No Observed Adverse Effect Level (NOAEL) was not established.

Dilley et al. (1982) investigated the short-term oral toxicity of a synthetic LAP (load, assemble, pack) munition plant wastewater mixture of α-TNT and RDX at a ratio of 1:0.62. A photolyzed mixture containing 10% RDX and 0.32% α-TNT by weight, was designated LAP(1). The dose levels ranged from 234 to 1190 mg/kg and 178 to 1780 mg/kg for Sprague-Dawley rats and Swiss-Webster mice administered LAP, respectively, and 250 to 1000 mg/kg for Swiss-Webster mice administered LAP(1); these LAP mixtures were dispersed uniformly in corn oil. The single-dose oral LD_{50}s for LAP were 947 (95% C.I. 707-1090) and 1130 (95% C.I. 946-1340) mg/kg in male and female mice, respectively, and 574 (95% C.I. 482-658) and 594 (95% C.I. 502-678) mg/kg in male and female rats, respectively. All deaths occurred within 24 hours of dosing; many animals exhibited aggressive behavior, depressed activity and discolored urine and many of those dosed at $\geq$ 790 mg/kg exhibited convulsions prior to death. Longer dispersion periods during preparation of the mixture lowered the LD_{50}s presumably because of increased dissolution of RDX, considered to be the more toxic component. The LD_{50}s for LAP(1) were 585 (95% C.I. 472-680) and 684 (95% C.I. 568-841) mg/kg for male and female mice, respectively. Deaths occurred between 0.5 and 1 hour of dosing; animals exhibited convulsions, squealing, depressed activity, and discolored urine.

Sprague-Dawley rats, Swiss-Webster mice and beagle dogs were treated in a repeated dose exposure study. Groups of 5 male and 5 female beagle dogs were dosed with gelatin capsules containing 0, 0.5, 5.0 or 50 mg LAP/kg/day. Several high-dose males exhibited convulsions following the second dose; one death resulted. Depressed body weight, depressed food intake, anemia and alterations in spleen (hemosiderosis), liver (hepatomegaly), and testes (atrophy) were exhibited at the high dose. The LOEL was 5.0 mg/kg/day based on signs of anemia; the NOEL was 0.5 mg/kg/day. Groups of 20 male and 20 female rats were

treated for 13 weeks with 0, 0.005, 0.05 or 0.5% LAP in the diet; mice were treated in the same manner with an additional group dosed at 0.25% LAP. The compound-related effects exhibited in rats and mice were similar to those found in dogs with the addition of uterine hypoplasia exhibited in female rats. Discolored urine, depressed activity and several biochemical changes were exhibited in all species. The NOEL for rats was 3.6 mg LAP/kg/day and that of mice was 8.3 mg LAP/kg/day, the lowest doses tested. A 4-week study in which irradiated LAP(1) was fed to rats at dosages of 0.003, 0.03 or 0.3% in the diet indicated that the irradiated mixture was less toxic than the unirradiated mixture.

b. Primary irritation and sensitization

In an acute dermal toxicity study, groups of five male and five female New Zealand albino rabbits received a single dermal application of 2 g RDX/kg in 1% carboxymethylcellulose solution; five additional males were retested. Animals were observed for 14 days; the toxicological endpoints included body weight measurements (on days 1, 3, 8, and 15) and observations of clinical signs and mortality. Except for a slight, transient loss in body weight, no other signs of toxicity were observed. Two males died on days 3 and 9; however, the cause of death (Tyzzer's disease) was unrelated to treatment. In the group of five retested males, there were no mortalities (Furedi-Machacek et al., 1984).

McNamara et al. (1974) studied the acute dermal toxicity of RDX (suspended in three solvents) in rabbits, guinea pigs (strain and sex not specified), and beagles. RDX was dissolved in DMSO, cyclohexanone, and acetone at concentrations of 33, 7.5, and 5.4% (w/v), respectively.

In a single-dose study, 1 mL of each mixture was applied to the shaved backs of six rabbits; controls received 1 mL of the solvent alone. All animals

were observed for skin irritation and systemic toxicity for 30 days. Blood samples drawn from each rabbit (time not specified) were analyzed for the following parameters: red blood cell count, white blood cell count, hematocrit, hemoglobin, alkaline phosphatase, serum glutamic-oxaloacetic transaminase (SGOT), blood urea nitrogen, creatinine, sodium, potassium, chloride, and carbon dioxide. Two treated rabbits and one control rabbit were sacrificed at 1 hour, 3 days, and 30 days after dosing for pathological evaluation. Topical applications of RDX in the three solvents did not cause skin irritation, deaths, systemic toxicity, or changes in any of the hematologic and blood chemistry parameters in treated rabbits. However, dermatitis was observed histologically in rabbits receiving RDX irrespective of the solvent. Dermatitis persisted for up to 30 days in rabbits treated with 33% RDX in DMSO.

In a repeated dose study, RDX (in the three solvents, as described above) was applied as a 0.1- or 1-mL dose to six rabbits/mixture/volume, 5 days/week for 4 weeks; controls received solvent alone. All animals were observed daily for 30 days, and blood samples were analyzed as described in the single-dose study. Pathological examination was conducted in one control and two treated rabbits sacrificed on days 7, 14, and 28. No skin irritation or changes in blood parameters were observed. One rabbit died after the fifth 0.1-mL dose, and one died after the tenth application of a 1.0-mL dose of 5.4% RDX in acetone. Another rabbit died after the eighth 1.0-mL application of 7.5% RDX in cyclohexanone. Histopathology revealed skin lesions at the site of RDX application that were characterized by a thickening or reddening and inflammation. Minimal dermatitis was observed in animals receiving the RDX mixtures as well as in some of the solvent controls. Rabbits treated with either 1, 10, or 20 1-mL doses of RDX in DMSO had dermatitis at necropsy, while the DMSO controls receiving the same doses did not develop dermatitis. Two animals that received

one 1.0-mL dose of RDX in acetone and two that received 20 1-mL doses of RDX in cyclohexanone developed dermatitis, and the solvent controls did not (McNamara et al., 1974).

Groups of four guinea pigs received a single topical application of a 3% (w/v) RDX/DMSO solution at doses of 316, 510, 1,000, or 2,000 mg/kg, or three applications at a dose of 1,000 mg/kg. Single doses of 316 or 510 mg/kg had no effect, while slight erythema was observed at the 1,000- or 2,000-mg/kg dose. When applied three times at 1,000 mg/kg, slight erythema was observed after the first application; later applications showed no further erythema. Applications of 2 mL DMSO alone produced no effects (McNamara et al., 1974).

Beagle dogs (sex not specified) received a single topical application of 1 mL of 33% RDX in DMSO (289.0 mg/kg), 7.5% RDX in cyclohexanone (65.7 mg/kg), or 5.4% RDX in acetone (47.3 mg/kg); each mixture was applied to two dogs. Two additional dogs received 480 mg/kg of 33% RDX in DMSO daily for 3 consecutive days. Treatment produced no consistent increase or decrease in any of the physiological parameters. One of the dogs at the 480-mg/kg dose was slightly more irritable and hyperactive for 20 to 30 minutes after the first and second applications; dogs appeared normal during the 2-week observation period (McNamara et al., 1974).

Groups of two to four beagles (sex not specified) received daily topical applications (on the dorsal area) of 1 mL of RDX in the three solvents (as described earlier), 5 days/week, for 4 weeks; control dogs received 1 mL of the solvent alone on the same schedule. Slight erythema and desquamation of the back was observed in some dogs (number not specified) during the second or third week of application of either DMSO alone or 289.0 mg/kg RDX in DMSO; no

effects were seen at a dose of 65.7 mg/kg RDX in cyclohexanone or 47.3 mg/kg RDX in acetone (McNamara et al., 1974).

McNamara et al. (1974) tested RDX for evidence of sensitization in male and female guinea pigs using topical or intradermal injections of RDX in the three solvents. During the sensitization phase, topical (0.5 mL) or intradermal (0.05 mL) doses were applied to the clipped dorsal thorax, 3 days/week for 3 weeks. Following a 2-week rest period, animals were challenged with a single topical or intradermal dose of RDX prepared in 1:1 (v/v) solvent-saline mixture (intradermal) and polyethylene glycol (topical). Although one route per animal was used for sensitization, both routes were used for challenge, one on each thigh at different times. Topical or intradermal injections of RDX in the three solvents produced no evidence of sensitization.

2. Longer-term Exposure

a. Ten- to 13-week studies

Schneider et al. (1978) dosed 30 Sprague-Dawley rats (sex not specified) orally with RDX in an isotonic saline slurry at 20 mg/kg/day for 90 days. A control group of 10 rats received isotonic saline on the same schedule. RDX did not induce convulsions or overt neurologic signs. Rats became lethargic, lost weight, developed rough hair coat, and had a blood-tinged exudate around the external nares immediately prior to death. During the study, eight dosed rats died (between days 42 and 77), apparently from exacerbation of chronic respiratory disease.

Cholakis et al. (1980) conducted 90-day subchronic dietary toxicity studies in male and female B6C3F$_1$ mice and Fischer 344 rats. RDX mixed in rodent laboratory chow was fed to the animals for 13 weeks; the RDX concentrations

were adjusted weekly based on the animals' body weight and food consumption. The RDX used in the study was contaminated with 9% HMX.

In the study with mice, groups of 10 male and 10 female mice, 5 weeks of age, received RDX doses of 10, 14, 20, 28, or 40 mg/kg/day for 13 weeks. A control group of 10 males and 10 females was given a normal diet. No meaningful changes were observed in clinical signs, mean body weight and food consumption, hematology, and clinical chemistry values or terminal organ weights in treated mice. Histopathology was not performed.

In a followup study (Cholakis et al., 1980), groups of 10 male and 12 female mice were fed 40, 60, or 80 mg/kg/day RDX in the diet for 2 weeks, followed by 80, 160, or 320 mg/kg/day RDX, respectively, for 11 weeks. An equal number of mice served as controls and received a normal diet. Animals were observed daily, and body weight and food consumption were measured weekly. Hematology and clinical chemistry tests were conducted at termination. Tissues from the control and high-dose groups were examined histopathologically.

Mortality occurred in 4 of 10 males (40%) and 2 of 12 females (16.7%) at the highest dose (320 mg/kg/day). One female died during week 6 of the study; the other mortalities occurred during week 11. No toxic signs were noted prior to death. Hyperactivity and/or nervousness were seen in 50% of the males at the highest dose during weeks 7 and 8; these signs were not observed in the females. Males fed 320 mg/kg/day RDX exhibited a significant ($p < 0.05$) increase in mean body weight gain during weeks 10, 12, and 13; females at this dose showed a 5.8% increase during week 13 when compared to controls. No changes were observed in mean food consumption during the 13-week period. Statistically significant ($p < 0.05$) changes were observed in some of the hematology parameters. Males fed 160 mg/kg/day RDX showed a 12% decrease in

erythrocyte count, a 7% decrease in hemoglobin concentration, and an 8% increase in mean corpuscular volume (MCV); however, there were no effects on these parameters at the higher dose (320 ppm). The mean corpuscular hemoglobin (MCHB) was increased 6 and 5% in females fed RDX at 80 or 160 mg/kg/day, respectively. This is not considered of toxicologic importance. In addition, intermittent changes in platelets and neutrophils (increased) and in serum glutamic-pyruvic transaminase (SGPT) (decreased) values were found in both sexes of treated mice. The effects of SGPT are not considered to be of toxicologic importance because the values were within the normal range and only increased values indicate an adverse effect.

Dose-related increases in mean absolute liver weight and mean liver weight relative to body weight and brain weight were seen in both sexes of mice; the increases were significant ($p < 0.05$) at 320 mg/kg/day. Histopathology revealed minimal focal myocardial degeneration (five males and two females), mild tubular nephrosis (four males and one female), and periportal hepatocellular vacuolization (five males) in the high-dose mice. Other tissue changes observed in treated mice were mild fatty infiltration and mild focal subscapular fibroplasia of the adrenals, mild microgranuloma, increased (minimal) karyomegaly of hepatocytes in the liver, and a dilated lumen of the uterus. The LOAEL for the study based on anemia in males was 160 mg/kg/day, and the NOAEL was 80 mg/kg/day.

In the 13-week study with Fischer 344 rats (Cholakis et al., 1980), 60 male and 60 female rats were divided into six groups, each consisting of 10 males and 10 females. Five groups were fed diets that provided an RDX intake of 10, 14, 20, 28, or 40 mg/kg/day. The sixth group, serving as controls, was given a normal diet. Rats were observed daily, and body weight and food consumption were measured weekly. Standard hematology tests were conducted at

30 days, 60 days, and at termination, and serum chemistry tests were conducted at termination. Tissues from controls and the two highest doses (28 and 40 mg/kg/day) were evaluated histopathologically.

No mortality occurred in the treated rats. The mean body weight of males fed RDX at 40 mg/kg/day was significantly ($p < 0.05$) lower than that of controls during weeks 2 to 13; at termination, the weight gain was 8.4% lower when compared to controls. Females exhibited dose-related decreases in body weight, the weight gain at termination was 5.5% lower in high-dose females than in controls. Mean food consumption was significantly ($p < 0.05$) reduced in males fed the high dose during weeks 1, 7, 10, 11, and 13; a slight, but nonsignificant, reduction in food consumption was observed in the females at this level.

In males fed 40 mg RDX/kg/day, significant ($p < 0.05$) decreases in hematocrit and hemoglobin (at 30 and 60 days) and increases in reticulocytes (at termination) were observed. In addition, a dose-related increase in platelet counts was observed at termination. After 30 days, females fed RDX at 28 mg/kg/day showed significant ($p < 0.05$) increases in reticulocytes and decreases in MCV and MCHB. The high-dose (40 mg/kg/day) females showed a significant ($p < 0.05$) increase in leukocytes. After 2 months, the reticulocytes in females fed 28 mg/kg/day returned to normal level, but the reticulocyte count decreased ($p < 0.05$) in females fed 40 mg RDX/kg/day. No significant changes were observed in any of the parameters for females at termination. Significant ($p < 0.05$) decreases were observed in glucose, SGPT, and serum potassium (males), and in SGOT and serum sodium (females) at termination; however, these values were reported to be within the normal historical ranges.

There was an apparent dose-related reduction in absolute and relative (to body weight and brain weight) terminal heart weight in both sexes fed 40

mg/kg/day RDX. The organ weight changes correlated with an increase in the incidence of myocardial degeneration (minimal) in females, 6/10 at the high dose compared with 2/10 in controls. In addition, there was an increased incidence (minimum degree) of liver portal inflammation in females receiving 40 mg/kg/day (7/10) as compared to controls (1/10). Based on anemia, the LOAEL was 28 mg/kg/day, and the NOAEL was 20 mg/kg/day.

Levine et al. (1981) fed RDX in the diet to groups of 10 male and 10 female Fischer 344 rats for 13 weeks at doses of 10, 30, 100, 300, or 600 mg/kg/day; a control group of 30 males and 30 females was given a normal diet. At termination, hematology and clinical chemistry tests were conducted, and all major organs (not specified) were weighed; 25 tissues from controls, from 30- and 100-mg/kg/day-dosed animals, and all major organs from the remaining groups were evaluated histologically. Male and female rats fed RDX at 300 or 600 mg/kg/day died within 1.5 weeks of dosing. The mean survival times were inversely correlated with the dose level, and there was no apparent sex difference. A dose-related and significant ($p \leq 0.05$) reduction in body weight gain was observed in males but not in females. Food consumption was significantly ($p \leq 0.05$) reduced in both sexes fed 100 mg/kg/day or above during study week 1, and in males (100 mg/kg/day) during study weeks 5 and 9. Dose-related and significant ($p \leq 0.05$) decreases in serum triglyceride levels were seen in both sexes; the levels were significantly ($p < 0.05$) lower than those of controls in the groups receiving 30 and 100 mg/kg/day. A slight but significant ($p \leq 0.05$) increase in leukocyte count was observed in females at all doses; however, this is of doubtful toxicologic importance because there were no effects on differential white blood cell ratios. Males showed slight increases in leukocyte counts, but the effect was not significant. No other hematology or serum chemistry changes were observed. The absolute and relative liver weights were significantly

($p \leq 0.05$) increased in females fed 100 mg/kg/day; however, histopathology indicated no treatment-related changes in the liver. Based on effects on liver weights, the LOAEL was 100 mg/kg/day and the NOAEL 30 mg/kg/day.

Von Oettingen et al. (1949) conducted longer term toxicity studies with RDX in rats (strain and sex not specified) and dogs (breed not specified). Groups of 15 rats received RDX in the feed at doses of 15, 50, or 100 mg/kg/day for 10 weeks. RDX concentrations were based on the weekly body weight and food consumption of the rats. No deaths occurred at the 15-mg/kg/day dose; however, the mortality rate was 60 and 86.6% in the 50- and 100-mg/kg/day dose groups, respectively. Most of the rats died during the first month. Necropsy revealed congestion of the lungs and gastrointestinal tract. Mean body weight of rats fed RDX at 50 or 100 mg/kg/day was lower than that of controls, while rats at the 15-mg/kg/day level showed normal weight gain. Hyperirritability and clonic/tonic convulsions were seen in rats fed 50 or 100 mg/kg/day RDX. No abnormal behavioral activity was seen at the lower dose. Based on CNS effects, the LOAEL was 50 mg/kg/day and the NOAEL was 15 mg/kg/day.

In a followup study, groups of 20 rats were administered RDX in the diet at doses to provide an intake of 15, 25, or 50 mg RDX/kg/day for 12 weeks; a control group of 20 rats received a normal diet. Mortality occurred in 1, 8, and 8 rats fed 15, 25, or 50 mg/kg/day, respectively. Necropsy revealed congestion of the lungs and gastrointestinal tract in most animals that died. The death in the low-dose group was not compound related. Body weight loss, hyperirritability, and clonic/tonic convulsions were observed in rats fed the two highest doses; these effects were not seen in rats fed 15 mg RDX/kg/day. Erythrocytes and hemoglobin content were reduced in all rats, including controls, during study weeks 3 and 4. However, normal values were observed in all rats by study week 8, except in rats fed 25 mg RDX/kg/day; recovery in these rats

was not complete until termination. No treatment-related changes were observed in rats sacrificed at termination (Von Oettingen et al., 1949). The LOAEL based on mortality and body weight loss was 25 mg/kg/day, and the NOAEL was 15 mg/kg/day.

In the study with dogs, seven healthy females were force-fed RDX (molded in a moistened pellet) at 50 mg/kg/day, 6 days/week for 6 weeks. One treated dog that died at the end of week 5 had many congested areas on the walls of the small intestines. Treatment caused hyperirritability, convulsions, and weight loss. The gross and microscopic changes in the organs and tissues were negligible (Von Oettingen et al., 1949).

Hart (1974) observed no signs of toxicity, except for temporary episodes of vomiting in beagles following dietary administration of RDX for 90 days. The vomiting only occurred sporadically and during the first 2 weeks. Groups of three male and three female dogs were fed RDX at 0.1, 1, or 10 mg/kg/day for 90 days; an equal number of control animals received a normal diet. All animals were observed daily, and body weights were recorded prior to initiation and at weekly intervals. Ophthalmoscopic examinations were conducted prior to initiation and at termination. Hematology, clinical chemistry, and urinalysis tests were performed on all dogs twice prior to initiation and during study weeks 4, 8, and 13. At necropsy, the heart, kidneys, liver, thyroids, adrenals, spleen, and testes (with epididymis) were weighed. Histopathological examinations of 12 selected tissues from the control and high-dose animals were conducted. No signs of toxicity, except for temporary episodes of emesis, were seen. Laboratory tests, ophthalmoscopic examination, and organ weights, as well as gross and microscopic examination, revealed no important differences from controls.

Martin and Hart (1974) conducted a 90-day subchronic oral toxicity study in cynomolgus monkeys. The animals ranged in age from 36 to 54 months; the males weighed 2.6 to 4.6 kg, and females weighed 2 to 4.2 kg. Groups of three male and three female monkeys were administered oral gavage doses of RDX in a 1% aqueous suspension of methylcellulose at 0.1, 1, or 10 mg/kg/day for 90 days. An equal number of monkeys, serving as controls, received a 1% aqueous suspension of methylcellulose alone on the same schedule. Animals were observed daily, and body weights were recorded prior to initiation and at weekly intervals. Ophthalmoscopic examinations were conducted prior to initiation and at termination. Standard hematology, clinical chemistry, and urinalysis tests were conducted before study initiation, at study weeks 5 and 9, and at termination. The bromosulphalein (BSP) liver function test was conducted twice before initiation and during study weeks 4, 8, and 13. Additionally, plasma levels of RDX were determined for each monkey at study weeks 5 and 9, and at termination. At necropsy, the thyroid, heart, liver, kidneys, and adrenals were weighed. Selected tissues and lesions from the control group and from the animals administered 10/mg/kg/day, and liver, kidneys, spleen, and gross lesions from the animals administered 0.1 and 1 mg/kg/day, were examined histopathologically. Vomiting and central nervous system disturbances were the major toxic signs observed in dosed monkeys. Vomiting occurred in one low-dose, three mid-dose, and five high-dose animals. Five of the six monkeys receiving 10 mg/kg/day showed 12 instances of CNS disturbances, usually involving tonic-type convulsions. One male animal at the high-dose level was sacrificed during study week 5 due to severe CNS disturbances. Eye examination, hematology and clinical chemistry parameters, urinalysis, BSP test, and organ weights showed no toxicologically significant changes. Body weights of treated and control monkeys were comparable. Histologic examination revealed necrotic and degenerative megakarocytes in bone marrow sections, and increased amounts of iron-positive material in liver cord

cytoplasm in monkeys given 10 mg RDX/kg/day. However, the authors were uncertain regarding the toxicological significance of these findings. Based on effects on the CNS, the LOAEL was 10 mg/kg/day and the NOAEL was 1 mg/kg/day.

b. Lifetime studies

Levine et al. (1983) evaluated the chronic effects of RDX in groups of 85 male and female Fisher 344 rats fed doses of 0.3, 1.5, 8.0, or 40.0 mg/kg/day for 24 months. RDX purity ranged from 89.2 to 98.7%, and particle size ranged from <22 μm (51.7%) to 440 μm. Mortality was increased in high-dose males and females throughout the study (e.g., 88 and 41%, respectively, at week 88 as compared to 32 and 33% for respective controls) (Table VI-2). Tremors and convulsions were frequently observed prior to deaths of high-dose males and females beginning at week 25. Behavioral hypersensitivity to stimuli resulted in fighting among cohabited high-dose males. Histologic evaluation failed to detect lesions of the central nervous system. The incidence of cataracts was significantly increased ($p < 0.05$) in high-dose females at weeks 78 and 104; the eyes of high-dose males appeared normal. These observations were considered to be treatment related.

Significant dose-related reductions ($p < 0.05$) in body weight gain were seen throughout the study for males receiving RDX at 8 mg/kg (5 to 6%) and 40 mg/kg (20 to 30%) when compared to controls. Females were less affected than males. Sporadic reductions in body weight gain were found in females receiving 8 mg/kg (5 to 6%) and 40 mg/kg (10 to 15%). Food consumption was slightly but significantly reduced for high-dose males at various intervals throughout the study; sporadic increases and decreases were reported for other dosed males and females.

Table VI-2. Representative Results of Mortality and Percent Survival in Rats Fed RDX in the Diet for 104 Weeks

Dose group (mg/kg/day)	Mortality (percent survival)[a] at week				
	26	52	66	88	104
			Males		
0	3 (96)	16 (79)	21 (72)	24 (68)	39 (48)
0.3	3 (96)	16 (79)	21 (72)	27 (64)	39 (48)
1.5	4 (95)	19 (75)	24 (68)	31 (59)	48 (36)
8.0	3 (96)	17 (77)	20 (73)	24 (68)	46 (39)
40.0	7 (91)	39 (48)	52 (31)	66 (12)	72 (4)
			Females		
0	3 (96)	18 (76)	22 (71)	25 (67)	36 (52)
0.3	3 (96)	17 (77)	20 (73)	23 (69)	33 (56)
1.5	3 (96)	16 (79)	22 (71)	25 (67)	35 (53)
8.0	4 (95)	17 (77)	21 (72)	23 (69)	36 (52)
40.0	3 (96)	22 (71)	28 (63)	31 (59)	49 (35)

[a] Percent survival was based on 75 rats/sex/group.

SOURCE: Adapted from Levine et al. (1983).

Hemoglobin and hematocrit values, and erythrocyte counts, were decreased in high-dose males and females throughout the study; males were affected to a greater extent than females. The study authors reported the anemia to be mild, since compensatory mechanisms (e.g., reticulocytosis) were not observed. Reticulocyte counts were not reported. Bone marrow was reported as appearing to be within normal limits. Spleens were reported to appear enlarged, with secondary splenic lesions in high-dose males and females (e.g., extramedullary hematopoiesis and sinusoidal congestion); however, spleen weights were not significantly increased. Individual pathology data were not available for review.

Hepatotoxicity, primarily at 40 mg/kg/day, was evidenced by hepatomegaly (although histological changes were not reported to be apparent), hypocholesteremia, hypotriglyceridemia, reduced serum albumin/total protein levels, and increased lactic dehydrogenase (LDH) levels. More specifically, cholesterol and triglyceride levels were significantly decreased in high-dose males and females throughout the study. Total protein and albumin were decreased in females receiving 8 and 40 mg/kg at weeks 52, 78, and 104; total protein was decreased in high-dose males at 52 weeks. LDH levels were increased in high-dose males at weeks 13 and 26 and decreased in mid- and high-dose females at week 26; LDH levels were not reported for weeks 52, 78, and 104. Absolute liver weights were increased for high-dose females, and relative liver weights were increased for high-dose males throughout the study.

Compound-induced renal toxicity was found primarily in high-dose males. Absolute kidney weights were significantly increased in high-dose females, and relative kidney weights were significantly increased in high-dose males throughout the study. Absolute and relative kidney weights were sporadically increased in males and females receiving 8 mg/kg. Blood urea nitrogen (BUN) levels were

significantly increased in high-dose females at week 104. Histologic examination of the kidneys showed renal medullary papillary necrosis in 15/19 males that received 40 mg/kg for more than 6 months and died by 12 months, but not in the same group sacrificed at 12 months or in any controls.

In high-dose males that died after 12 months or were sacrificed at termination, there was a significant ($p < 0.05$) increase in kidney lesions as medullary papillary necrosis, pyelitis, and uremic mineralization. Distension and cystitis of the bladder were also observed in high-dose males (Table VI-3). No corresponding changes were found in the urogenital system of females. Males receiving 1.5, 8.0, and 40.0 mg/kg/day exhibited increased pigment in the spleen (possibly a hematopoietic response and not adverse) and suppurative inflammation of the prostate (Table VI-3). The only significant ($p < 0.05$) histologic change in females was an increase in lenticular cataracts (32/48 in the high-dose group compared with 15/53 in controls).

Additional toxic effects found in high-dose males and females included hypoglycemia and thrombocytosis. Glucose levels were significantly decreased at this dose level throughout the study. White blood cell counts and platelet counts were increased in high-dose males and females throughout the study. Adrenals were found to be enlarged in males receiving 40 mg/kg/day, although histologic changes were not reported; adrenal weights were increased in high-dose males at 27 weeks and in high-dose females at 52 and 104 weeks. Absolute brain weights of high-dose females and relative brain weights of high-dose males were significantly increased throughout the study.

In summary, when RDX was fed at levels of 0.3, 1.5, 8.0, or 40.0 mg/kg/day to Fischer 344 rats for 24 months, major toxic effects included increased mortality, weight loss, hepatotoxicity, hypoglycemia, anemia with secondary splenic lesions, renal toxicity, urogenital lesions, testicular degeneration,

Table VI-3. Histologic Lesions in Male F-344 Rats Fed RDX for 2 Years[a]

Organ/lesion	Dose level (mg/kg/day)				
	0	0.3	1.5	8.0	40
Kidney	(55)[b]	(54)	(52)	(55)	(31)
Medullary papillary necrosis	0	1	0	0	18*
Pyelitis	0	1	0	1	5*
Uremic mineralization	1	1	2	0	13*
Bladder	(54)	(55)	(52)	(51)	(31)
Distension	0	2	1	3	25*
Cystitis	0	2	1	1	18*
Prostate	(54)	(55)	(52)	(55)	(31)
Suppurative inflammation	2	4	9*	12*	19*
Spleen	(55)	(52)	(52)	(55)	(31)
Increased pigment	3	1	11*	15*	18*

[a]Includes rats that died or were sacrificed moribund after 12 months and those sacrificed at termination.
[b]The numbers in parentheses are the numbers of animals for which a specific tissue was examined microscopically.
*Significantly different from control incidence, $p \leq 0.05$.

SOURCE: Levine et al. (1983).

CNS effects, and cataracts. Based on suppurative inflammation of the prostate of males receiving 1.5 mg/kg/day and above, the LOAEL was 1.5 mg/kg/day and the NOAEL was 0.3 mg/kg/day.

Hart (1977) fed RDX to male and female Sprague-Dawley rats at doses of 1.0, 3.1, or 10 mg/kg/day for 24 months. Survival was comparable to controls in high-dose males and females. Body weights in females receiving 3.1 and 10 mg/kg/day were significantly decreased ($p < 0.05$) from week 56 to week 88, nonsignificant decreases in body weights were seen in males receiving 10 mg/kg/day from week 60 to termination. The study authors, however, considered these findings to be toxicologically insignificant. Histopathologic evaluations were comparable between control and dosed animals. The LOAEL was determined to be 3.1 mg/kg/day and the NOAEL to be 1 mg/kg/day based on decreased body weights in females.

Lish et al. (1984) evaluated the chronic effects of RDX in groups of 85 male and female B6C3F$_1$ mice fed doses of 1.5, 7.0, 35.0, or 175 mg/kg/day for 24 months. RDX purity ranged from 89.2 to 98.7%; particle size ranged from <22 μm (51.7%) to 440 μm. The high dose was reduced from 175 mg/kg/day to 100 mg/kg/day during week 11 due to high mortality; mortality rates were 35 and 42% for high-dose males and females, respectively, at week 12 (Table VI-4). Survival was similar between dosed and control groups from week 12 to study termination. A high incidence of skin lesions (associated with fighting) was found in high-dose males during the first year of the study; study authors attributed this finding to treatment-related behavioral changes. During the second year, all dosed male and control groups exhibited this behavior; histology revealed chronic dermatitis and skin ulcers in all dosed males. This was reported to be a secondary treatment-related effect. Convulsions were exhibited

Table VI-4. Representative Results of Mortality and Percent Survival in Mice Fed RDX in the Diet for 104 Weeks

Dose group (mg/kg/day)	Mortality (percent survival)[a] at week				
	6	12	35	89	104
			Males		
0	0 (100)	1 (99)	11 (87)	32 (62)	39 (54)
1.5	0 (100)	0 (100)	13 (85)	34 (60)	43 (49)
7.0	0 (100)	0 (100)	13 (85)	31 (64)	44 (48)
35.0	0 (100)	0 (100)	13 (85)	33 (61)	48 (44)
100.0	18 (79)	30 (65)	46 (46)	60 (29)	63 (26)
			Females		
0	0 (100)	0 (100)	10 (88)	29 (66)	36 (58)
1.5	0 (100)	0 (100)	11 (87)	25 (71)	40 (53)
7.0	0 (100)	0 (100)	11 (87)	24 (73)	33 (61)
35.0	0 (100)	0 (100)	10 (88)	29 (66)	39 (54)
100.0	25 (71)	36 (58)	44 (48)	56 (34)	60 (29)

[a] Percent survival was based on 85 mice/sex/group.

SOURCE: Lish et al. (1984).

during month 24 in one male receiving 35 mg/kg and in one female receiving 100 mg/kg.

The body weight gains of high-dose females were significantly decreased ($p < 0.05$) from week 10 to study termination; the body weight gains of high-dose males were significantly decreased ($p < 0.05$) from study initiation to week 12 only. Sporadic increases and decreases were observed in food consumption throughout the study period. Slight but significant reductions in hemoglobin and hematocrit were found in high-dose females at week 53; however, RBC counts were increased at this test interval, and these effects on erythrocyte parameters were not reported at any other time during the study.

Large individual variations were found in clinical biochemistry data for dosed and control mice throughout the study. Hypercholesteremia was found in dosed males and females; elevated cholesterol levels were found earlier (week 14) and were more pronounced in females than in males. These increases were significant ($p < 0.05$) in high-dose females at the four intervals of measurement from week 14 to 79 and in high-dose males at weeks 26 and 53. Triglyceride levels were found to be significantly increased ($p < 0.05$) in females receiving 35 mg/kg at week 79 (high dose not tested) and nonsignificantly increased in all dosed females at week 105. Absolute and relative liver weights were found to be increased in high-dose males and females at interim sacrifices (weeks 26 and 53) and at study termination. The increases in absolute liver weights were significant ($p < 0.05$) at 53 and 105 weeks for females and at 105 weeks for males. Relative liver weight increases were significant ($p < 0.05$) at 26, 53, and 105 weeks in high-dose males and females. With the exception of hepatocellular tumors, which will be discussed in Section VI.B.5, Carcinogenicity, histopathologic lesions of the liver were not found.

Blood-urea-nitrogen (BUN) levels were slightly but significantly increased ($p < 0.05$) in high-dose females at week 14 and in 35-mg/kg females at week 79. Absolute kidney weights were found to be nonsignificantly increased at week 53 in 100-mg/kg males and signficantly increased ($p < 0.05$) in 35- and 100-mg/kg males at 105 weeks. Relative kidney weights were significantly increased ($p < 0.05$) at 26, 53, and 105 weeks in 100-mg/kg males and at 105 weeks in 35-mg/kg males. The incidence of renal tubular cytoplasmic vacuolization was reported to be increased in males at all dose levels when compared to controls at 26 weeks, however, the incidence of this lesion was increased in control males at 53 and 105 weeks and was comparable to dosed animals. This finding was reported to be compound related at 26 weeks. Additional toxic effects included significantly decreased ($p < 0.05$) brain weights in males receiving 100 mg/kg at week 53 and in females receiving 35 and 100 mg/kg at week 105. Enlarged hearts were found in high-dose males and females. The incidence of testicular degeneration in mice that died or were sacrificed moribund after 12 months and those sacrificed at study termination was 0/63, 2/60, 2/62, 6/59, and 3/27 at doses of 0, 1.5, 7.0, 35, and 100 mg/kg/day, respectively. The increase over controls was significant ($p < 0.05$) at the two highest doses. No increases in other nonneoplastic histologic lesions occurred in males or females.

In summary, when RDX was fed at levels of 1.5, 7.0, 35.0, or 100.0 mg/kg/day to B6C3F$_1$ mice for 24 months, major toxic effects included weight loss, increased liver weights, and testicular degeneration. Based on testicular degeneration in males, the LOAEL for systemic toxicity was 35 mg/kg/day, and the NOAEL was 7.0 mg/kg/day.

3. Reproductive Effects

Cholakis et al. (1980) conducted a two-generation reproduction study in Fischer 344 rats. The initial group of parental animals (F_0), consisting of 22

males and 22 females, was fed RDX in the diet at nominal doses of 0, 5, 16, or 50 mg/kg/day for 13 weeks. After treatment, the animals were mated and the dams were allowed to litter. After weaning, groups of 26 males and 26 females (F_1) were fed the same dietary concentrations of RDX as their parents for 13 weeks and were allowed to mate following completion of treatment. The dams were allowed to litter, and after weaning, male and female rats (F_2) were necropsied for histopathological examination. Parental toxicity was demonstrated at 50 mg/kg by increased F_0 mortality (18 versus 0% in the other groups) and significantly reduced body weights and food consumption. Transient reductions in body weight and food consumption were also noted at 5 and 16 mg/kg. Reproductive and developmental effects noted at 50 mg/kg were reduced mating and fertility rates and decreased litter size and pup survival. The numbers of live F_1 litters were 17, 21, 19, and 10 in the 0-, 5-, 6-, and 50-mg/kg groups, respectively; the respective mean litter sizes were 11.7, 13.8, 12.8, and 7.6. Only one high-dose litter survived to produce a second generation. Significantly reduced pup weights reflected developmental toxicity at both the 16- and 50-mg/kg dose levels; mean F_1 values at day 25 were 53, 47, 43, and 41 g in the 0-, 5-, 16-, and 50-mg/kg groups, respectively. The LOAEL for reproductive effects was 50 mg/kg/day, and the NOAEL was 16 mg/kg/day. The LOAEL for developmental toxicity was 16 mg/kg/day, and the NOAEL was 5 mg/kg/day.

4. Developmental Toxicity

Cholakis et al. (1980) conducted developmental toxicity studies in Fischer 344 rats and New Zealand rabbits. RDX did not induce a teratogenic effect. RDX was suggested to be teratogenic in the rabbits, but only at maternally toxic doses.

In the study with rats, groups of 24 or 25 rats received RDX, by gavage, at 0, 0.2, 2, or 20 mg/kg/day on days 6 through 19 of gestation. Maternal

toxicity was demonstrated at 20 mg/kg/day by mortality, hyperactivity, convulsions, reduced body weight gains (adjusted for gravid uterine weights), and decreased food consumption. Although one dam receiving 2.0 mg/kg/day had convulsions, Cholakis et al. (1980) reported no compound-related effects at 2.0 or 0.2 mg/kg/day. Embryotoxicity was demonstrated at 20 mg/kg/day by increased resorption rates. The means of postimplantation loss per litter were 6.8, 2.4, 5.1, and 18.6% at the 0-, 0.2-, 2.0-, and 20-mg/kg/day groups, respectively. Teratogenicity was not demonstrated at any of the dose levels tested. Based on embryotoxicity and maternal toxicity, the LOAEL was 20 mg/kg/day and the NOAEL was 2 mg/kg/day. This study is not suitable for calculation of a Ten-day Health Advisory due to the severity of the effects (convulsions) at 20 mg/kg/day.

In the study with rabbits, dams (number not reported) received RDX, by gavage, at levels of 0, 0.2, 2, or 20 mg/kg/day on days 7 through 29 of gestation, and 11 to 12 litters/group were delivered by cesarean section on day 30. Maternal toxicity was evidenced at 20 mg/kg/day by nonsignificantly reduced weight gains. Uterine implantation data and fetal weights were comparable for all groups; however, a nonsignificantly increased incidence of fetal abnormalities (e.g., spina bifida, meningocele, misshapen/enlarged eye bulges, gastroschisis, limb reductional defects, tail abnormalities) suggested developmental toxicity at 20 mg/kg. Cleft palate was not found on gross examination of 94 to 110 fetuses in 11 to 12 litters at doses of 0.2, 2.0, or 20 mg/kg/day, but it was found on soft tissue examination in 1/46 fetuses at 0.2 mg/kg/day and in 2/44 and 2/52 fetuses in the 2.0- and 20.0-mg/kg/day groups, respectively. No cleft palates were found in controls; the normal control incidence in rabbits is about 0.1%. Spina bifida was found in 3/110 fetuses (two litters) from dams dosed at 20.0 mg/kg/day; this anomaly was not found in controls or at the two lower doses. No maternal or developmental effects were noted at 0.2 or 2.0

mg/kg/day (Cholakis et al., 1980). Thus, based on maternal toxicity and developmental effects, the LOAEL was 20 mg/kg/day and the NOAEL was 2.0 mg/kg/day.

Angerhofer et al. (1986) investigated the developmental toxic effects of RDX in rats. The authors conducted a range-finding study using groups of six pregnant rats treated with RDX, by gavage, at dose levels of 0, 10, 20, 40, 80, or 120 mg/kg/day on gestational days 6 through 15. Treatments with 40, 80, and 120 mg/kg/day were lethal in all females. Animals in the 20-mg/kg/day group exhibited urogenital and nasal discharge throughout the dosing period; no signs of compound-related toxicity were noted at 10 mg/kg/day. Fetuses from the groups dosed with RDX had significantly reduced body weights when compared with controls. The teratogenicity study consisted of 39, 40, 40, and 51 mated females dosed with RDX on gestational days 6 through 15 at 0, 2, 6, and 20 mg/kg/day, respectively. A total of 0, 1, 1, and 16 animals, respectively, died in the above groups. No clinical signs were noted for the animals dosed at 2 and 6 mg/kg/day; however, antemortem signs for animals in the 20-mg/kg/day group included urogenital discharge, nasal and oral exudate, convulsions, and prostration. Necropsy findings did not provide evidence of specific causes for the deaths of these animals.

Among the surviving animals, maternal body weights for the high-dose group were significantly ($p \leq 0.05$) reduced when compared with controls at gestational days 10, 13, and 16; however, body weights in this group were comparable with those of controls on gestation day 20.

The incidence of fetal resorptions was slightly increased for all groups dosed with RDX, but these fetal effects were not significantly different from those of controls and the effects were not dose related. Fetal weights and lengths were reported to be significantly ($p < 0.05$) reduced at all dose levels

when compared with controls. However, when statistical analysis was repeated on the data, using the litter as the basis, it was found that fetal weight and length were significantly decreased compared to controls only in fetuses from dams dosed at 20 mg/kg/day (Table VI-5). The male-to-female ratios were 0.83, 0.88, 0.94, and 0.99 for the 0-, 2-, 6-, and 20-mg/kg/day groups, respectively. No teratogenic effects were noted in this study. Based on maternal deaths and toxicity and a decrease in fetal weight and fetal length, the LOAEL is 20 mg/kg/day and the NOAEL is 6 mg/kg/day.

5. Carcinogenicity

RDX was not found to be carcinogenic when fed to rats (two strains); however, it was found to produce hepatocellular and alveolar/bronchiolar carcinomas and adenomas when fed to mice.

Levine et al. (1983) evaluated the incidence of tumor formation in male and female Fischer 344 rats fed RDX in the diet at doses of 0.3, 1.5, 8.0, or 40.0 mg/kg/day for 24 months. Following treatment, animals were examined for occurrence of tumors in various organs/systems. RDX was not found to be carcinogenic in rats at doses up to 40.0 mg/kg/day. This study is reviewed in detail in Section VI.B.2.b., Lifetime Studies.

RDX was not found to be oncogenic in male and female Sprague-Dawley rats fed RDX in the diet at doses of 1.0, 3.1, or 10 mg/kg/day for 24 months (Hart, 1977). The incidence of neoplasms was found to be comparable between dosed and control animals.

Lish et al. (1984) evaluated the incidence of tumors in groups of 85 male and female B6C3F_1 mice fed RDX at doses of 1.5, 7.0, 35.0, or 100 mg/kg/day for 24 months (Table VI-6 and Table VI-7, respectively). The incidence of

Table VI-5. Fetal Weight and Length (± SD) in Progeny of Rats Administered RDX

Dose level	Weight (g)	Length (cm)
0	3.81±0.47	3.71±0.16
2	3.70±0.39	3.68±0.17
6	3.72±0.34	3.67±0.15
20	3.45±0.43*	3.52±0.21*

*Significantly different from control value using analysis of variance and Duncan's test for multiple comparisons, $p < 0.05$; the analysis is on a litter basis.

SOURCE: Adapted from Angerhofer et al. (1986).

Table VI-6. Incidence of Neoplastic Lesions in Male B6C3F$_1$ Mice Fed RDX in the Diet for 24 Months

Organ/finding[a]	Dose level (mg/kg/day)				
	0	1.5	7.0	35.0	100.0
Liver	(63)[b]	(60)	(62)	(59)	(27)
Malignant lymphoma	0 (0%)[c]	1 (1.7%)	4 (6.4%)	5 (8.5%)	0 (0%)
Hepatocellular carcinoma	13 (20.6%)	20 (33.3%)	16 (25.8%)	18 (30.5%)	6 (22.2%)
Hepatocellular adenoma	8 (12.7%)	6 (10.0%)	1* (1.6%)	7 (11.9%)	7 (25.9%)
Hepatocellular adenoma and carcinoma combined	21 (33.3%)	26 (43.3%)	17 (27.4%)	25 (42.4%)	13 (48.1%)
Lung	(63)	(60)	(62)	(59)	(27)
Alveolar/bronchiolar carcinoma	3 (4.8%)	6 (10.0%)	3 (4.8%)	7 (11.9%)	5 (18.5%)
Alveolar/bronchiolar adenoma	6 (9.5%)	5 (8.3%)	5 (8.1%)	7 (11.9%)	1 (3.7%)
Alveolar/bronchiolar adenoma and carcinoma combined	9 (14.3%)	11 (18.3%)	8 (12.9%)	14 (23.7%)	6 (22.2%)
Kidney	(63)	(60)	(62)	(59)	(27)
Malignant lymphoma	1 (1.6%)	2 (3.3%)	4 (6.4%)	4 (6.8%)	1 (3.7%)

[a]Includes animals sacrificed at study termination and those that died or were sacrificed moribund in the course of the study.
[b]Number of animals with specific tissue examined histologically.
[c]Percentage of incidence.
*Significantly different from control value ($p < 0.05$).

SOURCE: Adapted from Lish et al. (1984).

Table VI-7. Incidence of Neoplastic Lesions in Female B6C3F$_1$ Mice Fed RDX in the Diet for 24 Months

Organ/finding[a]	Dose level (mg/kg/day) 0	1.5	7.0	35.0	100.0
Liver	(65)[b]	(62)	(64)	(64)	(31)
Hepatocellular carcinoma	0 (0%)[c]	4 (6.4%)	3 (4.7%)	6 (9.4%)	3 (9.7%)
Hepatocellular adenoma	1 (1.5%)	1 (1.6%)	6 (9.4%)	6 (9.4%)	3 (9.7%)
Hepatocellular adenoma and carcinoma combined	1** (1.5%)	5 (8.1%)	9* (14.1%)	12* (18.8%)	6* (19.4%)
Lung	(65)	(62)	(64)	(64)	(31)
Alveolar/bronchiolar carcinoma	3 (4.6%)	1 (1.6%)	3 (4.7%)	3 (4.7%)	4 (12.9%)
Alveolar/bronchiolar adenoma	4 (9.2%)	2 (3.2%)	5 (7.8%)	9 (14.1%)	3 (9.7%)
Alveolar/bronchiolar adenoma and carcinoma combined	7 (10.8%)	3 (4.8%)	8 (12.5%)	12 (18.8%)	7 (22.6%)

[a]Includes animals sacrificed at study termination and those that died or were sacrificed moribund in the course of the study.
[b]Number of animals with specific tissue examined histologically.
[c]Percentage of incidence.
*Significantly different from control value ($p < 0.05$), Fisher's Exact test.
**Significant trend ($p \leq 0.05$), Cochran-Armitage test; analysis by reviewers.

SOURCE: Adapted from Lish et al. (1984).

hepatocellular carcinomas and adenomas combined was reported to be significantly increased ($p < 0.05$) in females receiving RDX at 7.0, 35.0, and 100.0 mg/kg (14.1, 18.8, and 19.4%, respectively) when compared to concurrent (1.5%) or historical (7.9%) controls (Tables VI-7 and VI-8). These findings were considered to be compound related in females. In addition, it was reported that the incidence of hepatocellular adenomas in females receiving 7.0 and 35.0 but not 100 mg/kg/day was significantly ($p < 0.05$) increased when compared to historical controls. Historically, the incidence of combined hepatocellular adenomas and carcinomas in untreated male $B6C3F_1$ mice is 31.1% as compared to 7.9% for untreated females of this strain (Table VI-8) (Haseman et al., 1984). The incidence of combined hepatocellular adenomas and carcinomas in high-dose males was 48.1% as compared to 33.3% for the concurrent control group; the increase was not significant (Table VI-6). A nonsignificant increase in alveolar and bronchiolar carcinomas was found in high-dose males and females; histiocytes were reported to be increased in the lungs of high-dose females. Malignant lymphoma of the kidney was reported to be slightly increased (nonsignificantly) in males receiving RDX at levels of 1.5, 7.0, and 35.0 mg/kg (3.3, 6.4, and 6.8%, respectively) when compared with concurrent controls (1.6%). This finding was not seen in female mice. In summary, the incidence of hepatocellular carcinomas and adenomas (combined) was significantly increased in RDX-treated female mice. Based on EPA Guidelines for Carcinogen Risk Assessment, RDX is classified in Group C: Possible Human Carcinogen.

This study had some technical flaws. The high dose was lowered from 175 mg/kg/day to 100 mg/kg/day during week 11 due to high mortality in both sexes. This mortality substantially reduced the number of high-dose animals at risk (see Table VI-4). Since there were four doses in the study, the high dose could be removed for quantitation purposes and still provide an acceptable

Table VI-8. Incidence of Primary Liver Tumors in Historical Control B6C3F$_1$ Mice

Finding	Tumor incidence	
	Males	Females
Number of tissues examined	(2,334)	(2,469)
Hepatocellular carcinoma	498 (21.3%)	101 (4.1%)
Hepatocellular adenoma	240 (10.3%)	98 (4.0%)
Hepatocellular adenoma and carcinoma combined	725 (31.1%)	196 (7.9%)

SOURCE: Adapted from Haseman et al. (1984).

potency estimate. The RDX used contained 3 to 10% HMX. The increases in hepatocellular adenomas or carcinomas were not significantly ($p > 0.05$) increased in dosed females when analyzed separately but were significant when combined.

6. Genotoxicity

RDX gave negative results in all genotoxicity studies conducted.

The mutagenic potential of RDX was evaluated in two studies using the Salmonella/microsomal preincubation assay. Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 were exposed to RDX at concentrations of 1, 10, 100, 300, or 1,000 ug/plate in one study (Cholakis et al., 1980), and at 0.625 or 1.25 mg/plate in the second study (Whong et al., 1980). The assays were conducted with and without metabolic activation (S-9 fraction from livers of Aroclor-induced rats). In both studies, RDX was nonmutagenic.

Simmon et al. (1977) tested the mutagenicity of several munitions wastewater chemicals before and after chlorination or ozone treatment. RDX was evaluated in Salmonella strains TA98, TA100, TA1535, TA1537, and TA1538 at several concentrations ranging from 0.24 to 14 ug/plate, and in Saccharomyces cerevisiae strain D3 at concentrations ranging from 0.00004 to 0.0023%. The assays were conducted with or without metabolic activation (S-9 fraction from livers of Aroclor-induced rats). RDX was nonmutagenic before and after chlorination in these assays.

In a dominant lethal assay, male Fischer 344 rats were fed RDX-containing diets providing 0, 5, 16, or 50 mg/kg/day for 13 weeks and then mated with virgin females. RDX did not induce a dominant lethal effect. There was no evidence of a preimplantation loss of blastocysts or postimplantation of embryos (Cholakis et al., 1980).

RDX gave negative results in an unscheduled DNA synthesis assay using WI-38 (human fibroblasts) when tested at a maximum concentration of 4,000 μg/mL, with or without metabolic activation (Dilley et al., 1978).

7. Neurotoxicity

MacPhail et al. (1985) studied neurobehavioral effects after acute or subchronic oral administration of RDX to Sprague-Dawley rats. In acute studies, RDX in corn oil was administered, by gavage, in doses of 12.5, 25, or 50 mg/kg. Testing for behavioral effects was conducted 2 and 24 hours after administration. In subchronic studies, doses of 1, 3, or 10 mg/kg/day were given for 30 days, and testing was performed prior to dosing and after 16 and 30 days of dosing. Between 24 and 64 rats of each sex were studied in a series of neurobehavioral tests. After acute dosing, a wide range of sensory, motor, and cognitive effects were found in the rats, but no overt neurotoxic effects were found. Little evidence of behavioral toxicity was observed after subchronic dosing.

Motor activity was tested in a figure-8 maze with blind alleys that converged on a central open area; photoelectric cells monitored total activity as well as temporal and spatial distribution of motor activity. In the acute study, there was a significant dose-related decrease in activity (all doses) with some indication of a residual effect at 24 hours. Landing footspread, measured in the same groups of rats, was consistently decreased at 2 hours, but no dose-related effect was observed. In an acoustical startle response test, a 13-kHz startle stimulus was presented 10 times in each testing session in the presence of three different background noise intensities. A dose-related increase in response latency was observed after single doses of RDX (MacPhail et al., 1985).

In a schedule-controlled behavior test, male rats were trained to press a key to deliver sweetened condensed milk in response to a light cue over the milk dipper. RDX administration decreased the overall operant response rate and, at the highest dose, recovery to baseline performance did not occur until 3 days after dosing. There was a dose-response effect on recovery. In a flavor aversion conditioning test, RDX-treated rats preferred water to a saccharin-flavored solution.

In the subchronic studies, no clear behavioral effects were observed in any of the above tests after daily dosing at levels of up to 10 mg/kg/day.

Burdette et al. (1988) studied spontaneous seizure activity, audiogenic seizure activity, and the phenomenon of kindling in Long-Evans male rats after oral administration of RDX. Oral gavage doses of RDX (class 5 particle size) dispersed in carboxymethylcellulose by ultrasonic treatment were administered to groups of 10 to 12 rats, and the animals were observed for 8 hours. Spontaneous convulsions occurred in about 20% of the rats dosed at 12.5 mg/kg RDX within 2 hours and subsided by 4 hours. At 25- and 50-mg/kg doses, spontaneous convulsions occurred in 80% of the animals and seizure activity persisted for 2 and 6 hours, respectively. The threshold plasma level of RDX at seizure was 3.1 µg/mL. Susceptibility to audiogenic seizures, elicited by exposure to an ultrasonic source, was significantly increased with dose and persisted for up to 300 seconds after a 60-mg/kg dose of RDX; this dose resulted in death of 6 of 16 rats.

To study the kindling phenomenon, electrodes were implanted in the amydaloid nucleus of the brain. Kindling is characterized by development of seizure activity by repeated daily electric stimulation of the amydaloid at a current that is a fraction of the normal threshold current for seizures.

RDX given at nonconvulsive daily doses of 6 mg/kg/day acted as a chemical kindling agent. Convulsions could be induced by low currents a week after cessation of RDX dosing, indicating that the effect was not due to accumulation of RDX in the body. The results indicated to Burdette et al. (1988) that the primary target for RDX toxicity may be the limbic system of the brain.

VII. HEALTH ADVISORY DEVELOPMENT

A. SUMMARY OF HEALTH EFFECTS DATA

In humans, the major toxic effects of RDX have been on the central nervous system (CNS). Exposure of workers in a munitions plant via inhalation of dust containing RDX has resulted in nausea, irritability, convulsions, unconsciousness, and amnesia (Kaplan et al., 1965). Military personnel have been exposed to RDX and its decomposition products while burning composition C-4 explosives in the field to heat food; inhalation of the smoke resulted in clonic/tonic convulsions (Ketel and Hughes, 1972; Hollander and Colbach, 1969). Ingestion of RDX has caused similar CNS effects (Stone et al., 1969, Woody et al., 1986).

Acute toxicity studies by Cholakis et al. (1980) indicated oral LD_{50} values of about 80 mg/kg in mice and 118 mg/kg in rats. It has been estimated that convulsions occur in rats when plasma levels reach 13.8 µg/mL (Schneider et al., 1977).

Subchronic 90-day feeding studies in mice and rats indicate effects on the blood and liver. In both sexes of mice, increased liver weights were seen in groups receiving RDX at 320 mg/kg/day, and anemia was seen in males receiving 160 mg/kg/day (Cholakis et al., 1980). In rats, anemia was seen at a dose level of 28 mg/kg/day in males (Cholakis et al., 1980), and increased liver weight was seen at a dose level of 100 mg/kg/day in females (Levine et al., 1981). In a 10-day oral gavage study in monkeys, vomiting and convulsions were seen in five of six animals receiving RDX at 10 mg/kg/day, but no central nervous system effects were seen at 1 mg/kg/day (Martin and Hart, 1974).

Lifetime feeding studies in rats and mice produced CNS effects, increased mortality, weight loss, anemia, hepatotoxicity, renal toxicity, testicular degeneration, and inflammation of the prostate. In male and female rats fed RDX in the diet at a level to give a daily intake of 40 mg/kg, tremors and convulsions, increased mortality, and enlargement of the liver were observed. Anemia and enlargement of the kidneys accompanied by histologic changes were also found in males receiving 40 mg/kg/day. Inflammation of the prostate was found at 1.5, 8, and 40 mg/kg/day, no effects were noted at a dose of 0.3 mg/kg/day (Levine et al., 1983). In a study in mice by Lish et al. (1984), increased mortality was seen in the first 10 weeks of the study when mice received 175 mg/kg/day. The high dose was reduced to 100 mg/kg/day. Decreased weight gain was observed in females receiving 100 mg RDX/kg/day between 10 weeks and study termination. Increased liver weights were found in males and females receiving 100 mg/kg/day. The males receiving 35 or 100 mg/kg/day exhibited testicular degeneration. No important toxic effects were found at 7 mg/kg/day.

RDX was not found to be mutagenic in bacteria (Whong et al., 1980, Simmon et al., 1977). It gave negative results in the dominant-lethal test (Cholakis et al., 1980) and in an unscheduled DNA synthesis assay (Dilley et al., 1978).

RDX was not carcinogenic in rats (Levine et al., 1983; Hart, 1977). In $B6C3F_1$ mice, there was a significant increase in the incidence of hepatocellular carcinomas and adenomas (combined) in females receiving 7, 35, or 100 mg/kg/day for 2 years. Mortality in mice receiving the highest dose was excessive and the dose was lowered from 175 to 100 mg/kg at week 11. RDX is classified as Group C: Possible Human Carcinogen.

In a two-generation reproduction study in rats, decreased fertility was observed at 50 mg/kg/day. Developmental effects (decreased pup weights) were seen at 16 and 50 mg/kg/day; no effects were observed at 5 mg/kg/day (Cholakis et al., 1980). RDX was found to be embryotoxic in rats at 20 mg/kg/day (Cholakis et al., 1980; Angernofer et al., 1986) but was not found to be teratogenic.

B. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Health Advisories are generally determined for One-day, Ten-day, Longer-term (approximately 7 years), and Lifetime exposures if adequate data are available that identify a sensitive noncarcinogenic endpoint of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(NOAEL \text{ or } LOAEL)\ (BW)}{(UF)\ (\_\_\_\_\ L/day)} = \_\_\_\_ \text{ mg/L } (\_\_\_\_ \text{ µg/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg BW/day).

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100 or 1,000), in accordance with NAS/ODW guidelines.

L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

1. One-day Health Advisory

In humans, RDX intoxication results in central nervous system effects such as convulsions and unconsciousness. Good quantitative data on the minimum dose that causes the effects are not available. One study on acute intoxication of a 3-year-old child who consumed RDX measured blood and spinal fluid levels of RDX

and estimated the dose ingested (Woody et al., 1986). However, since assumptions were made to estimate the dose, and a single individual was exposed, this study was judged to have inadequate data for HA development. In a study in dogs, a single intravenous dose of 3.37 mg RDX/kg was reported to cause an erratic electroencephalogram (EEG) pattern and decreased blood pressure. However, graphic data for this dose (the lowest dose tested) were not presented, only one dog was tested, a NOAEL was not established, and the route of exposure may not have been appropriate for setting a One-day HA value for RDX.

Since these data were not judged suitable for determining a One-day HA value for RDX, it is recommended that the Longer-term HA for a 10-kg child (0.1 mg/L) be used as a conservative estimate for the One-day HA value.

2. Ten-day Health Advisory

No data found in the available literature were suitable for determination of the Ten-day HA value for RDX. It is therefore recommended that the Longer-term HA for a 10-kg child (0.1 mg/L) be used as a conservative estimate for the Ten-day HA value.

3. Longer-term Health Advisory

In a 90-day study in cynomolgus monkeys, convulsions occurred in five of six animals receiving RDX, by gavage, at 10 mg/kg/day. No CNS effects were seen in three males and three females receiving 1 mg/kg/day (Martin and Hart, 1974). Based on this study, the LOAEL is 10 mg/kg/day and the NOAEL is 1 mg/kg/day. In rats, the LOAEL for CNS effects was 25 mg/kg/day and the NOAEL was 15 mg/kg/day in a 90-day feeding study (Von Oettingen et al., 1949). LOAELs and NOAELs were available for effects on liver weight and liver pathology in mice (Cholakis et al., 1980) and in rats (Levine et al., 1981), but they

were not considered because the values were much less sensitive than those for CNS effects in monkeys. The Cholakis et al. (1980) developmental toxicity study was considered but rejected due to the severity of the effects (convulsions) at the LOAEL (20 mg/kg/day).

The Longer-term HA for the 10-kg child is calculated as follows:

$$\frac{(1 \text{ mg/kg/day})\ (10 \text{ kg})}{(1 \text{ L/day})\ (100)} = 0.1 \text{ mg/L } (100 \text{ µg/L})$$

where:

1 mg/kg/day = NOAEL, based on CNS effects in monkeys following 90-day oral dosing at 10 mg RDX/kg/day.

10 kg = assumed weight of a child.

1 L/day = assumed water consumption of a 10-kg child.

100 = uncertainty factor, chosen in accordance with ODW/NAS guidelines using a NOAEL from an animal study.

The Longer-term HA for a 70-kg adult is calculated as follows:

$$\frac{(1 \text{ mg/kg/day})\ (70 \text{ kg})}{(2 \text{ L/day})\ (100)} = 0.35 \text{ mg/L (rounded to 400 µg/L)}$$

where:

1 mg/kg/day = NOAEL, based on CNS effects in monkeys following 90-day oral dosing at 10 mg RDX/kg/day.

70 kg = assumed weight of an adult.

2 L/day = assumed water consumption of a 70-kg adult.

4. Lifetime Health Advisory

The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic

adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). The RfD is an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL IS A MEDIUM-SPECIFIC (I.E., DRINKING WATER) LIFETIME EXPOSURE LEVEL, ASSUMING 100% EXPOSURE FROM THAT MEDIUM, AT WHICH ADVERSE, NONCARCINOGENIC HEALTH EFFECTS WOULD NOT BE EXPECTED TO OCCUR. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. THE LIFETIME HA IS DETERMINED IN STEP 3 BY FACTORING IN OTHER SOURCES OF EXPOSURE, THE RELATIVE SOURCE CONTRIBUTION (RSC). THE RSC FROM DRINKING WATER IS BASED ON ACTUAL EXPOSURE DATA OR, IF DATA ARE NOT AVAILABLE, A VALUE OF 20% IS ASSUMED. If the contaminant is classified as a Group A or B carcinogen, according to the Agency's classification scheme of carcinogenic potential (U.S. EPA, 1986), then caution should be exercised in accessing the risks associated with lifetime exposure to this chemical.

Three 24-month continuous feeding studies, one in $B6C3F_1$ mice and two in rats (F-344 and Sprague-Dawley), were considered. The LOAELs and NOAELs established are presented in Table VII-1.

In the study with rats by Levine et al. (1983), F-344 rats were fed doses of RDX at 0.3, 1.5, 8.0, or 40 mg/kg/day for 24 months. Based on a dose-related increase in the incidence of suppurative inflammation of the prostate of males receiving 1.5 mg/kg/day and above, the LOAEL is 1.5 mg/kg/day and the NOAEL is 0.3 mg/kg/day. This study is the most sensitive for deriving a DWEL.

Table VII-1. Summary of Candidate Studies for Derivation of the Drinking Water Equivalent Level (DWEL) for RDX

Species/ strain	Route	Duration	Endpoint	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Rat/F-344	Oral/ diet	Lifetime	Inflammation of prostate	0.3	1.5	Levine et al. (1983)
Rat/S-D	Oral/ diet	Lifetime	Decreased body weight in females	1.0	3.1	Hart et al. (1977)
Mouse/ $B6C3F_1$	Oral/ diet	Lifetime	Testicular degeneration	7.0*	35	Lish et al. (1984)

*The NOAEL is 7.0 mg/kg/day for systemic effects based on the absence of systemic effects at this dose and the separation by EPA of tumorigenic and systemic changes; it is recognized that hepatocellular adenomas and carcinomas were exhibited in female mice at this dose level.

In a 24-month feeding study in Sprague-Dawley rats (Hart et al., 1977), no histologic changes were seen at the doses tested (1, 3.1, and 10 mg/kg/day). However, significant decreases were seen in body weight gain in females at 3.1 and 10 mg/kg/day. Thus, a NOAEL of 1.0 mg/kg/day was determined.

In a 2-year feeding study in $B6C3F_1$ mice, an increase in testicular degeneration was found in males receiving 35 mg/kg/day but not in those receiving a dose of 1.5 or 7.0 mg/kg/day (Lish et al., 1984). There was an increase in absolute and relative liver weights in males and females administered 100 mg/kg/day and an increase in combined hepatocellular tumors in females receiving 7.0, 35, or 100 mg/kg/day.

Data on pharmacokinetics in humans are inadequate, and no data are available for mice. Therefore, it cannot be determined which species most closely resembles man in absorption, metabolism, and excretion of RDX.

The study by Levine et al. (1983) has been selected for calculation of the DWEL, since it has the lowest NOAEL (0.3 mg/kg/day) among all the studies.

Using this study, the DWEL is derived as follows:

Step 1. Determination of the Reference Dose (RfD)

$$RfD = \frac{(0.3\ mg/kg/day)}{100} = 0.003\ mg/kg/day$$

where:

0.3 mg/kg/day = NOAEL, based on increased incidence of suppurative inflammation in the prostate of males receiving 1.5 mg/kg/day.

100 = the uncertainty factor, chosen in accordance with ODW/NAS guidelines using a NOAEL from a chronic animal study (10X for intraspecies variation and 10X for interspecies variation).

Step 2. Determination of the Drinking Water Equivalent Level (DWEL)

$$DWEL = \frac{(0.003\ mg/kg/day)\ (70\ kg)}{2\ L/day} = 0.105\ mg/L \text{ (rounded to 100 } \mu g/L)$$

where:

0.003 mg/kg/day = RfD.

70 kg = assumed body weight of an adult.

2 L = assumed daily water consumption of an adult.

Step 3. Determination of Lifetime Health Advisory

$$\text{Lifetime HA} = \frac{(0.105\ mg/L)\ (0.2)}{10} = 0.002\ mg/L\ (2\ \mu g/L)$$

where:

0.105 mg/L = Drinking Water Equivalent Level (DWEL).

0.2 = Assumed Relative Source Contribution (RSC) if actual data are not available.

10 = ODW policy, additional factor of 10 to account for equivocal evidence of carcinogenicity for Group C chemicals.

C. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The carcinogenic potential of RDX has been evaluated in Fischer 344 rats (Levine et al., 1983), Sprague-Dawley rats (Hart, 1976) and B6C3F1 mice (Lish et al., 1984). RDX was not found to be carcinogenic when fed to the rats (both strains). However, it was found to produce significant combined hepatocellular adenomas/carcinomas in B6C3F1 female mice. RDX is classified Group C: Possible Human Carcinogen.

RDX was not found to be oncogenic in male and female Sprague-Dawley rats fed RDX in the diet at doses of 1.0, 3.1, or 10 mg/kg/day for 24 months (Hart, 1976).

Levine et al. (1983) evaluated the carcinogenicity of RDX in male and female Fischer 344 rats fed doses of 0.3, 1.5, 8.0, or 40.0 mg/kg/day RDX for 24 months. The major toxic effects observed included anemia with secondary splenic lesions, hepatotoxicity and urogenital lesions. Based on adverse systemic effects at 1.5 mg/kg/day, the MTD was achieved.

Lish et al. (1984) evaluated the incidence of tumors in groups of 85 male and female B6C3F1 mice fed doses of 1.5, 7.0, 35.0, or 100 mg/kg/day RDX for 24 months. The incidence of hepatocellular carcinomas and adenomas combined was significantly increased ($p < 0.05$) in females receiving 7.0, 35.0, and 100.0 mg RDX/kg (14.1, 18.8, and 19.4%, respectively) when compared to concurrent (1.5%) or historical (7.9%) controls. These findings were considered to be compound-related in females. Historically, the incidence of combined hepatocellular adenomas and carcinomas in untreated male B6C3F1 mice is 31.6% as compared to 7.9% for untreated females of this strain (Haseman et al., 1984). In addition, the incidence of hepatocellular adenoma in females receiving 7.0 and 35.0 but not 100 mg/kg/day was significantly ($p < 0.05$) increased when compared to historical controls.

The incidence of combined hepatocellular ademomas and carcinomas in high-dose males was 48.1% as compared to 33.3% for the concurrent control group; the increase was not significant. A nonsignificant increase in alveolar and bronchiolar carcinomas was found in high-dose males and females; histocytes were reported to be increased in the lungs of high-dose females. Malignant lymphoma of the kidney was reported to be slightly increased (nonsignificant) in males receiving 1.5, 7.0, and 35.0 mg/kg when compared with concurrent controls. This finding was not seen in female mice.

This study had some technical flaws which may have compromised its sensitivity to evaluate oncogenicity. The high-dose was lowered from 175 mg/kg/day to 100 mg/kg/day during week 11 due to high mortality in both sexes. This mortality substantially reduced the number of high-dose animals. The RDX used contained 3 to 10% HMX. The increase in hepatocellular adenomas or carcinomas were not significantly ($p > 0.05$) increased (when compared to concurrent controls) in dosed females when analyzed separately but only when combined.

DOSE-RESPONSE DATA (CARCINOGENICITY, ORAL EXPOSURE)

Tumor Type -- liver
hepatocellular carcinoma
and adenomas (combined)

Test Animals -- mice/B6C3F1/females

Route -- diet/oral

Reference -- Lish et al. (1984)

Dose		
Administered (mg/kg/day)	Human* Equivalent (mg/kg/day)	Tumor Incidence
0.0	0.0	1/65
1.5	0.13	5/62
7.0	0.58	9/64
35.0	2.90	12/64
100.0	8.30	6/31

* Administered dose $\div$ (70 kg/.040 kg).33

The human equivalent dose was determined using a standard surface area correction factor. The animal study dose is divided by the ratio of the human weight (70 kg) to the mouse weight (.040 kg) raised to the 1/3 power. The high-dose data was not used in the slope factor calculation since it was lowered from 175 mg/kg/day to 100 mg/kg/day during week 11. The unit risk should not

be used if the water concentration exceeds 3,000 µg/L. Above this concentration the slope factor may differ from that stated.

SUMMARY OF RISK ESTIMATES

	Without High-dose
Oral Slope Factor $(mg/kg/day)^{-1}$	1.1E-1
Drinking Water Unit (µg/L) Risk	3.1E-6
Extrapolation Method	Linearized Multistage Procedure, extra risk

Drinking Water Concentrations at Specific Risk Levels:

Risk Level	Concentrations µg/L)
E-4 (1 in 10,000)	30.0
E-5 (1 in 100,000)	3.0
E-6 (1 in 1,000,000)	.3

The multistage model was used for high-to-low dose extrapolation (Crump and Watson, 1979; Howe and Crump, 1982). Global83 was used to fit the data in the experimental dose range and to obtain upper 95% confidence limits on risk. The multistage model conforms to a biological model of tumor initiation and promotion (Crump et al., 1977) and provided an adequate fit to the dose-response data for RDX. The relationship of the concentration (µg/L) of a chemical in drinking water to cancer risk is expressed as follows:

$$\frac{35000}{q_1^*} \times R = C$$

Where:

q_1^* = $(mg/kg/day)^{-1}$, human slope factor

R = (10^{-4}, 10^{-5}, 10^{-6}, etc.)

C = concentration of chemical in µg/L

35000 = conversion factor for mg to µg and exposure assumption that a 70 kg adult drinks 2L of water/day

DISCUSSION OF CONFIDENCE (CARCINOGENICITY, ORAL EXPOSURE)

There are three animal bioassay studies to evaluate the carcinogenic potential of RDX. Both well conducted rat studies (Fischer 344 rat and Sprague-Dawley rat) were clearly negative. The RDX was found to be carcinogenic in the female B6C3F1 mice. This study had some previously discussed technical problems but is clearly positive for liver carcinomas and adenomas combined.

OTHER CANCER RISK MODELS

For comparison purposes the drinking water concentration associated with an excess cancer risk of 10^{-6} was 0.3 µg/L for the One-hit model. The drinking water concentrations associated with an excess cancer risk of 10^{-6} for the other models (Multihit, Logit, Probit, and Weibull) were all <0.002 µg/L. The estimates for these models were calculated with RISK81 (Kovar and Krewski, 1981).

VIII. OTHER CRITERIA, GUIDANCE, AND STANDARDS

The threshold limit value (TLV) for RDX recommended by the American Conference of Government Industrial Hygienists is 1.5 mg/m^3 (notation skin) (ACGIH, 1986), and the short-term exposure limit (STEL) is 3 mg/m^3. OSHA has also recommended a TLV of 1.5 mg/m^3 (National Research Council, 1982).

Schneider et al. (1978) suggested an ingestion limit of 0.1 mg/kg/day or 2 to 3 ppm in potable water.

In 1980, the United States Army Medical Bioengineering Research and Development Laboratory recommended an RDX limit of 0.03 mg/L in drinking water as an interim standard (National Research Council, 1982).

IX. ANALYTICAL METHODS

Published methods for analyzing RDX fall into two broad categories: analysis of bulk material and trace analysis. Volumetric methods involving reduction, hydrolysis, or acid-base titrations are used for bulk analysis. Methods for analyzing trace quantities (below 10 ppm) of RDX include the following: thin-layer chromatography (TLC), high-pressure liquid chromatography (HPLC), gas-liquid chromatography (GLC), and single-sweep polarography.

Polarography has a detection limit of 0.05 mg/L for RDX, an analysis time of approximately 5 minutes, and reproducibility of ± 10%. However, metal ions and some anions can interfere with the analysis. Since these interferences have not been completely characterized, polarography is not routinely used for RDX analysis (Whitnach, 1976, as cited in Sullivan et al., 1979).

Since the detection limit for RDX using TLC is 25 mg/L for direct determination (Leach and Hash, 1972, as cited in Sullivan et al., 1979), TLC is generally not used for quantitative determination of RDX (Sullivan et al., 1979). TLC may be useful for qualitative identification of RDX when it is present in mixtures with other munitions components (Sullivan et al., 1979).

GLC is one of the two preferred analytical techniques for determination of RDX at low concentrations (below 10 ppm). Each of the GLC methods involves extraction of RDX into a solvent prior to determination. Hoffsomer et al. (1977, as cited in Sullivan et al., 1979) extracted RDX with benzene/acetone followed by GLC determination using an electron capture (EC) detector; the detection limit for the method is 0.002 ppm. Jain (1976, as cited in Sullivan et al., 1979) used benzene as the extraction solvent and either an EC or a flame ionization detector (FID); the detection limit was 0.03 ppm. Sullivan

et al. (1977, as cited in Sullivan et al., 1979) used ethyl acetate as the extraction solvent and either EC or an alkaline earth FID; the detection limits were 0.005 ppm for water samples and 0.2 ppm for sediment samples. One disadvantage of the GLC methods is that other, less volatile munition components (e.g., HMX) that may be present in the same sample cannot be codetermined (Sullivan et al., 1979).

HPLC methods would appear to be the methods of choice for detection of RDX at low concentrations. Even though the detection limits are not quite as low for HPLC (0.05 to 0.4 ppm) as for GLC (0.002 to 0.2 ppm), advantages include low operating temperatures and determination of RDX with less volatile components. The HPLC methods have the following features in common: solvent extraction, an aqueous methanol mobile phase, and use of an ultraviolet (UV) detector. Hale et al. (1978, as cited in Sullivan et al., 1979) obtained a detection limit of 0.05 ppm for RDX in both soil and water samples using ether extraction, a mobile phase of 30% methanol in water, Zorbax-ODS stationary phase, and detection at 230 nm. Spanggord et al. (1978, as cited in Sullivan et al., 1979) determined RDX levels in "load, assemble, and pack" (LAP) wastewater using a mobile phase of 60% methanol:40% water and a stationary phase of M-Bondapak L_{18}; detection limits were 0.4 ppm at 254 nm and 0.2 ppm at 210 nm. Stilwell et al. (1977) determined RDX in wastewater from the Holston Army Ammunition Plant following extraction with ethyl acetate. A mobile phase of 40% methanol:60% water was used with a stationary phase of Partisil 10-ODS; the detection limit was 0.05 ppm at 230 nm. HMX was determined concurrently with a detection limit of 0.05 ppm.

X. TREATMENT TECHNOLOGIES

Noss and Chyrek (1984) studied the degradation of RDX using ultraviolet radiation, hydrogen peroxide addition, and ultrasound cavitation. Hydrogen peroxide alone had no effect on munitions degradation. Similarly, ultrasound cavitational processes yielded no benefit when used alone or when combined with other treatments. Hydrogen peroxide applied at initial concentrations less than 0.01% enhanced RDX decomposition by ultraviolet photolysis. During treatment with ultraviolet radiation in combination with 0.01% hydrogen peroxide, RDX (18.9 mg/L) was degraded rapidly (half-life = 8.0 minutes).

The use of an ultraviolet light-hydrogen peroxide system for treatment of RDX in wastewater was studied by the Naval Weapons Support Center (ESR, 1985). RDX and its organic reaction products were completely destroyed using a system with 0.05 to 0.15% hydrogen peroxide and a minimum of 10 megawatt-minutes of ultraviolet light at 254 nm/mole of explosive. Kubose and Hoffsommer (1977, as cited in Sullivan et al., 1979) have shown that concentrations of 20 to 40 mg RDX/L in aqueous solution could be reduced 98% by photolysis using the full spectral output from a medium-pressure mercury vapor lamp (220 nm to 1367 nm) with irradiation periods of 15 seconds.

Kubose and Hoffsommer (1977, as cited in Sullivan et al., 1979) have indicated that strongly basic ion exchange resins may be a potential single-step waste treatment method for the degradation of RDX. This process involves RDX adsorption onto the resin and reaction with quaternary ammonium hydroxide. Sullivan et al. (1979) commented that this procedure is only feasible for aqueous solutions that have fairly low acid and/or anion concentrations.

Chemical oxidation using potassium dichromate, potassium permanganate, or calcium hypochlorate will destroy nitramines. Jackson et al. (1976, as cited in Sullivan et al., 1979) found that concentrations of $Ca(OCl)_2$, $K_2Cr_2O_7$, or $KMnO_4$ at 1,000 mg/L completely oxidized 50 mg RDX/L within 72, 48, or 24 hours, respectively.

Chemical coagulation using lime was also studied by Jackson et al. (1976, as cited in Sullivan et al., 1979). Ninety percent RDX removal was achieved using a 500-mg/L lime dose. Castorina et al. (1977, as cited in Sullivan et al., 1979) have cautioned against the use of polymer flocculants because of incompatibilities with RDX.

Jackson et al. (1976, as cited in Sullivan et al., 1979) reported that complete adsorptive removal of RDX (1.5 to 12 mg/L) was achieved using activated charcoal and Rohm and Haas XAD-2 resins. Activated carbon was found to be superior to resin at a hydraulic loading of 10 gpm/ft^3. Vlahakis (1974, as cited in Sullivan et al., 1979) found that activated carbon had a capacity of 125 mg RDX/g carbon for RDX alone and 76 mg RDX/g carbon when in the presence of TNT.

Data regarding aerobic microbiological degradation of RDX are mixed. Pilot plant studies by Green (1972, as cited in Sullivan et al., 1979) suggested that RDX could be at least partially degraded by aquatic microflora. Green was able to remove 42% of the RDX from manufacturing wastes using an activated sludge reactor dominated by Pseudomonas and Alcaligenes. Conversely, Soli (1973) concluded that aerobic heterotrophs could not degrade RDX.

McCormick et al. (1981) reported that RDX is biodegraded under anaerobic conditions, yielding the following products: hexahydro-1-nitroso-3,5-dinitro-

1,3,5-triazine; hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine; hexahydro-1,3,5-trinitroso-1,3,5-triazine; hydrazine; 1,1-dimethylhydrazine; 1,2-dimethylhydrazine; formaldehyde, and methanol. Jackson et al. (1976, as cited in Sullivan et al., 1979) achieved 100% removal of RDX by anaerobic fermentation from waters containing 50 mg RDX/L in conjunction with supplemental carbon sources: sucrose, methanol, and hydroxyethyl cellulose. Times required for complete removal of RDX ranged from 1 day for hydroxyethyl cellulose-supplemented wastewater to 9 days for sucrose-supplemented water.

XI. CONCLUSIONS AND RECOMMENDATIONS

Based on the available animal data and on adverse effects on the central nervous system of monkeys administered RDX by oral gavage for 90 days, the Longer-term HA for a 10-kg child has been determined to be 0.1 mg/L (100 µg/L). In the absence of adequate animal data to determine a One-day or Ten-day Health Advisory, the Longer-term HA for a 10-kg child, 0.1 mg/L (100 µg/L), is used as a conservative estimate of the One-day or Ten-day HA. The Longer-term HA for a 70-kg adult was determined to be 0.35 mg/L (400 µg/L). A Lifetime HA of 0.002 mg/L (2 µg/L) for a 70-kg adult has been determined, based on a Drinking Water Equivalent Level (DWEL) of 0.100 mg/L (100 µg/L). The DWEL is based on a Reference Dose of 0.003 mg/kg/day where the effect was suppurative inflammation of the prostate of male rats fed RDX for 2 years. Based on the study by Lish et al. (1984), RDX is classified as Group C: Possible Human Carcinogen. The classification of RDX in EPA Group C is based upon limited animal data. A quantitative cancer risk assessment, based on the limited animal data, is provided to support selection of the uncertainty factors for the recommended lifetime HA.

A comparison report "Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for RDX" (Appendix A) summarizes the scope of existing data reviewed for this HA. This comparison report delineates the areas where additional data and/or a clarification of existing data would be appropriate.

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APPENDIX A

Data Deficiencies/Problem Areas and Recommendations for Additional Data Base Development for RDX

DATA BASE DEVELOPMENT

A. OBJECTIVES

The objective of this document is to provide an evaluation of data deficiencies and/or problem areas encountered in the review process for RDX and to make recommendations, as appropriate, for additional data base development. This document is presented as an independent analysis of the current status of RDX toxicology, as related to its possible presence in drinking water, and includes a summary of the background information used in development of the Health Advisory (HA). For greater detail on the toxicology of RDX, the Health Advisory on RDX should be consulted.

B. BACKGROUND

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a white crystalline solid that has been extensively used in military munitions formulations. It is relatively insoluble in water but can be expected to be found in wastewater from RDX-manufacturing operations. RDX can be removed from water by the use of ion exchange resins or charcoal and can be decomposed by ultraviolet photolysis in the presence of hydrogen peroxide. It can be biodegraded by anaerobic fermentation (Sullivan et al., 1979).

The pharmacokinetic properties of RDX have been extensively studied in rats. Schneider et al. (1977, 1978) found that, in rats, RDX was completely absorbed via the oral route and the rate of absorption was determined by the fineness of the powder in the slurry administered. The rate of gastrointestinal absorption in rats is faster than that in miniature swine or humans; in rats, peak plasma levels are reached in 2 to 3 hours, whereas in swine and probably in humans, plasma levels peak approximately 12 hours after

dosing. Absorbed RDX is rapidly cleared from the plasma and distributed to tissues. When drinking water saturated with RDX (7.5 mg/L) was offered to rats for 90 days, there was no accumulation of RDX in the tissues. The half-lives of clearance of RDX from plasma are of a similar order of magnitude in rats and humans. The $t_{1/2}$ was 10.1 hours in rats (Schneider et al., 1977) and was 15.1 hours in the one available human study (Woody et al., 1986). RDX is metabolized by the liver, and its metabolites are excreted primarily in the urine. The metabolites have not been identified and characterized.

In humans, the toxic effects of RDX have been on the central nervous system (CNS). Exposure of workers in a munitions plant via inhalation of dust containing RDX has resulted in nausea, irritability, convulsions, unconciousness, and amnesia (Kaplan et al., 1965). Military personnel have been exposed to RDX while burning composition C-4 explosive in the field to heat food; inhalation of the smoke resulted in clonic/tonic convulsions (Ketel and Hughes, 1972; Hollander and Colbach, 1969). Ingestion of RDX has caused similar CNS effects (Stone et al., 1969; Woody et al., 1985, 1986).

Acute toxicity studies by Cholakis et al. (1980) indicated oral LD_{50} values of about 80 mg/kg in mice and 118 mg/kg in rats. Intravenous administration of single doses of RDX to beagle dogs caused convulsions and death at a dose of 40 mg/kg, central nervous system hyperactivity and nonlethal convulsions at a dose of 20 mg/kg, and decreased blood pressure and erratic electroencephalographic patterns at doses of 3.37 and 6.78 mg/kg (McNamara et al., 1974).

Subchronic 90-day feeding studies in mice and rats indicate effects on the blood and liver. In both sexes of mice, increased liver weights were observed in groups receiving 320 mg/kg/day, and anemia was seen in males receiving 160 mg/kg/day (Cholakis et al., 1980). In rats, anemia was observed at a dose level

of 28 mg/kg/day in males (Cholakis et al., 1980), and increased liver weight was seen at a dose level of 100 mg/kg/day in females (Levine et al., 1981). In a 10-day oral gavage study in monkeys, vomiting and convulsions occurred in five of six animals at 10 mg/kg/day, but no central nervous system effects were seen at 1 mg/kg/day (Martin and Hart, 1974).

Lifetime feeding studies in rats and mice produced CNS effects, increased mortality, weight loss, anemia, hepatotoxicity, renal toxicity, testicular degeneration, and inflammation of the prostate. In male and female rats fed RDX in the diet at a level to give a daily intake of 40 mg/kg, tremors and convulsions, increased mortality, and enlargement of the liver were observed. Anemia and enlargement of the kidneys accompanied by histologic changes were also found in males receiving 40 mg/kg/day. Inflammation of the prostate was found at 1.5, 8, and 40 mg/kg/day; no effects were noted at a dose of 0.3 mg/kg/day (Levine et al., 1983). In a study in mice by Lish et al. (1984), increased mortality was seen in the first 10 weeks of the study when mice received 175 mg/kg/day. The high dose was reduced to 100 mg/kg/day. Decreased weight gain was seen in females receiving RDX at 100 mg/kg/day between 10 weeks postadministration and study termination. Increased liver weights were found in males and females receiving 100 mg/kg/day. The males receiving 35 or 100 mg/kg/day exhibited testicular degeneration. No important toxic effects were found at 7 mg/kg/day.

RDX was not found to be mutagenic in bacteria (Whong et al., 1980; Simmon et al., 1977). It gave negative results in the dominant-lethal test (Cholakis et al., 1980) and in an unscheduled DNA synthesis assay (Dilley et al., 1978).

RDX was not carcinogenic in rats (Levine et al., 1983; Hart, 1977). In $B6C3F_1$ mice, a significant increase was observed in the incidence of

hepatocellular carcinomas and adenomas (combined) in females receiving 7, 35, or 100 mg/kg/day for 2 years. However, mortality in mice receiving the highest dose was excessive, and the dose was lowered from 175 to 100 mg/kg at week 11. RDX is classified as Group C: Possible Human Carcinogen.

In a two-generation reproduction study in rats, decreased fertility was observed at 50 mg/kg/day. Developmental effects (decreased pup weights) were seen at 16 and 50 mg/kg/day; no effects were observed at 5 mg/kg/day (Cholakis et al., 1980). RDX was found to be embryotoxic in rats at 20 mg/kg/day (Cholakis et al., 1980; Angerhofer et al., 1986) but was not found to be teratogenic. In a study in rabbits, RDX caused maternal toxicity at 20 mg/kg/day, and there was suggestive evidence for a teratogenic effect at 2 and 20 mg/kg/day (Cholakis et al., 1980).

Based on the findings in these studies, One-day and Ten-day HA values were established at 0.1 mg/L, and the Longer-term HA value was established for an adult at 0.35 mg/L (400 µg/L). The Lifetime HA for an adult is 0.002 mg RDX/L (2 µg/L).

Methods of chemical analysis utilizing extraction and gas-liquid chromatography reviewed by Sullivan et al. (1979) are adequate for detection of RDX at low concentration in water. RDX in wastewater can be degraded by ultraviolet irradiation in the presence of hydrogen peroxide (ESB, 1985). Other methods using ion exchange resins or charcoal have been reviewed by Sullivan et al. (1979).

C. DISCUSSION

Available data on the pharmacokinetics, health effects, analysis, and treatment of RDX have been reviewed.

Pharmacokinetic studies in rats indicate that RDX is effectively absorbed via the oral route, and it is rapidly metabolized and excreted. Available data indicate that the rate of clearance in humans is approximately of the same order of magnitude as that in rats. Further studies in animals are unlikely to yield additional data pertinent to the development of HA values.

Available acute toxicity studies of RDX include oral LD_{50}s in mice and rats and a study in dogs using the intravenous route, in which a LOAEL for nonlethal CNS effects was established but a NOAEL was not achieved. Six longer term studies (90 days) were available in mice, rats, dogs, and monkeys. In five of these studies, LOAELs and NOAELs were established for various endpoints: CNS effects, anemia, liver weight changes, and histologic effects on the liver. Three lifetime studies were available, two in rats and one in mice; LOAELs and NOAELs were established. Additional short-term, longer term, and lifetime studies are not likely to be pertinent in development of HA values. A carcinogenicity study in mice was flawed because the high dose exceeded the maximum tolerated dose (MTD) and was lowered from 175 to 100 mg/kg/day at 11 weeks. Although mortality was high at this dose, sufficient animals were available to assess carcinogenicity (Lish et al., 1984). There was a carcinogenic response, but time-to-tumor could not be analyzed from the available data. The animals with hepatocellular tumors could be identified from individual animal histopathology tables, but the identification numbers for histopathology did not correspond with the animal numbers on the disposition tabulation.

A two-generation reproduction study and several mutagenicity studies were available. Not all categories of genotoxicity were investigated. A mutagenicity test in a eukaryotic microorganism with and without activation and a test for chromosome aberrations and sister chromatid exchange would fulfill this data

gap. Two studies on developmental toxicity in rats were available that indicated embryotoxic, but not teratogenic, effects. A study in rabbits (Cholakis et al., 1980) gave results that suggested a possible teratogenic response. Cleft palate was seen on soft tissue analysis of fetuses from dosed females. However, it was not recorded on gross examination or found in the fetuses examined for skeletal anomalies. It is recommended that a teratology study be repeated in rabbits using 20 pregnant dams/dose group to reevaluate the teratogenic potential of RDX.

The methods for analysis of RDX in wastewater appear to be adequate to detect levels that may be considered hazardous to health. The methods of treating RDX-contaminated water appear to be adequate.

E. CONCLUSIONS/RECOMMENDATIONS

Based on the above discussion, the following conclusions/recommendations can be made:

1. The available studies on RDX toxicity are generally considered adequate for development of Health Advisories useful in dealing with the potential contamination of drinking water.

2. It is recommended that a teratology study in rabbits be repeated. Additional genotoxicity studies in eukaryotes and a test for chromosome aberrations are recommended to fill data gaps in genotoxicity.

3. Additional carcinogenicity bioassays should be considered to clarify the carcinogenic potential of RDX.

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