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Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Executive Summary

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

RDX is a synthetic chemical used primarily as a military explosive. RDX releases have been reported in air, water, and soil, and exposure is likely limited to individuals in or around military facilities where RDX is or was produced, used, or stored. Oral exposure may occur from drinking contaminated groundwater or ingesting crops irrigated with contaminated water. Inhalation or dermal exposures are more likely in occupational settings.

Epidemiological studies provide only limited information on worker populations exposed to RDX; several case reports describe effects primarily in the nervous system following acute exposure to RDX. Animal studies of ingested RDX demonstrate toxicity, including effects on the nervous system, urinary system (kidney and bladder), and prostate.

Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on evidence of positive trends in liver and lung tumor incidence in experimental animals. There are no data on the carcinogenicity of RDX in humans.

ES.1. EVIDENCE FOR HAZARDS OTHER THAN CANCER: ORAL EXPOSURE

Nervous system effects are a human hazard of RDX exposure. Several human case reports and animal studies provide consistent evidence of an association between RDX exposure and effects on the nervous system, including findings related to the induction of seizures, abnormal electrical activity, convulsions, tremors, and a reduced threshold for seizure induction by other stimuli; behavioral effects that may be related to seizures such as hyperirritability, hyper-reactivity, and other behavioral changes. Mechanistic data support the hypothesis that RDX-induced seizures and related behavioral effects likely result from inhibition of gamma-aminobutyric acid (GABA)ergic signaling in the limbic system. Some investigators reported that unscheduled deaths in experimental animals exposed to RDX were frequently preceded by convulsions or seizures.

Urinary system effects are a potential human hazard of RDX exposure based largely on observations of histopathological changes in the kidney and urinary bladder of male rats exposed to RDX at doses higher than those associated with nervous system effects. The available evidence indicates that male rats are more sensitive than females, and rats are more sensitive than mice to RDX-related urinary system toxicity. There is suggestive evidence of male prostate effects associated with RDX exposure based on an increased incidence of suppurative prostatitis in male rats exposed to RDX in the diet for 2 years, in one of the few studies that evaluated the prostate. There is no known mode of action (MOA) for effects of RDX exposure on the urinary system or prostate, although there are studies indicating GABA helps regulate urinary system and prostate

function. Evidence for effects on other organs/systems, or developmental effects, was more limited than for the endpoints summarized above.

ES.1.1. Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ-specific RfDs were derived for hazards associated with RDX exposure (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Table ES-1. Organ/system-specific reference doses (RfDs) and overall RfD for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Effect	Basis	RfD (mg/kg-day)	Study exposure description	Confidence
Nervous system	Convulsions	4×10^{-3}	Subchronic	Medium
Urinary system	Kidney medullary papillary necrosis	1×10^{-2}	Chronic	Medium
Prostate	Suppurative prostatitis	8×10^{-4}	Chronic	Low
Overall RfD	**Nervous system effects**	**4×10^{-3}**	**Subchronic**	**Medium**

The overall RfD (see Table ES-2) is derived to be protective of all types of hazards associated with RDX exposure. Although the RfD for prostate effects results in a smaller value, it was not selected as the overall RfD due to uncertainties in the evaluation of this endpoint ("low confidence"). The effect of RDX on the nervous system was chosen as the basis for the overall RfD because nervous system effects were observed most consistently across studies, species, and exposure durations, and because they represent a sensitive human hazard of RDX exposure. Evidence for effects of RDX on the urinary system and prostate is more limited relative to the effects of RDX on the nervous system. Incidence of seizures or convulsions as reported in a subchronic gavage study (Crouse et al., 2006) was selected for deriving the overall RfD because this endpoint was measured in a study that was well conducted, used a test material of high purity (99.99%), and had five closely spaced dose groups that supported characterization of the dose-response curve. In contrast, most other studies used a technical grade with ~10% or more impurities. Benchmark dose (BMD) modeling was used to derive the point of departure (POD) for RfD derivation (expressed as the lower confidence limit on the benchmark dose [$BMDL_{05}$]). A 5% response level was chosen because of the severity of the endpoint.

Table ES-2. Summary of reference dose (RfD) derivation

Critical effect	Point of departure[a]	UF	Chronic RfD	Confidence
Nervous system effects (convulsions) 90-d F344 rat study Crouse et al. (2006)	$BMDL_{05-HED}$: 1.3 mg/kg-d	300	4×10^{-3} mg/kg-d	Medium

AUC = area under the curve; BMDL = benchmark dose lower confidence limit.
[a]A benchmark response (BMR) of 5% was used to derive the BMD and BMDL. The resulting POD was converted to a $BMDL_{05-HED}$ using a PBPK model based on modeled arterial blood concentration. The concentration was derived from the AUC of modeled RDX concentration in arterial blood, which reflects the average blood RDX concentration for the exposure duration normalized to 24 hr.

A PBPK model was used to extrapolate the $BMDL_{05}$ derived from a rat study to a human equivalent dose (HED) based on RDX arterial blood concentration, which was then used for RfD derivation.

The overall RfD, 4×10^{-3} mg/kg-day, was calculated by dividing the $BMDL_{05}$ expressed as a human equivalent dose ($BMDL_{05-HED}$) for nervous system effects by a composite uncertainty factor (UF) of 300 to account for extrapolation from animals to humans (3), interindividual differences in human susceptibility (10), and uncertainty in the database (10).

Because a subchronic-to-chronic uncertainty factor (UF_S) of 1 was applied to the POD based on evidence that nervous system effects (in particular convulsions) are more strongly driven by dose than duration of exposure, the RfD may be appropriate for assessing health risks of less-than-lifetime as well as chronic durations of exposure.

The overall confidence in the RfD is medium based on high confidence in the principal study (Crouse et al., 2006) and medium to low confidence in the database. Confidence in the database is reduced largely because of (1) differences in test material used across studies (i.e., differences in formulation and particle size that may have affected RDX absorption and subsequent toxicity), (2) uncertainties in the influence of oral dosing methods (in particular, based on evidence that bolus dosing of RDX resulting from gavage administration induces neurotoxicity at doses lower than administration in the diet), and (3) significant limitations in the available studies to fully characterize subconvulsive neurological effects as well as developmental neurotoxicity.

ES.2. EVIDENCE FOR HAZARDS OTHER THAN CANCER: INHALATION EXPOSURE

No studies were identified that provided useful information on the effects observed following inhalation exposure to RDX. Of the available human epidemiological studies of RDX, none provided data that could be used for dose-response analysis of inhalation exposures. The single experimental animal study involving inhalation exposure is not publicly available and was excluded from consideration due to significant study limitations, including small numbers of animals tested, lack of controls, and incomplete reporting of exposure levels. Therefore, the available health effects

literature does not support the identification of hazards following inhalation exposure to RDX nor the derivation of an inhalation reference concentration (RfC).

While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support development of an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an RfC from the RfD.

ES.3. EVIDENCE FOR HUMAN CARCINOGENICITY

Under EPA's cancer guidelines (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for RDX. RDX induced benign and malignant tumors in the liver and lungs of mice (Parker et al., 2006; Lish et al., 1984) or rats (Levine et al., 1983) following long-term administration in the diet. The potential for carcinogenicity applies to all routes of human exposure.

ES.4. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

A quantitative estimate of carcinogenic risk from oral exposure to RDX was based on the increased incidence of hepatocellular adenomas or carcinomas and alveolar/bronchiolar adenomas or carcinomas in female B6C3F$_1$ mice observed in the carcinogenicity bioassay in mice (Lish et al., 1984). This 2-year dietary study included four dose groups and a control group, adequate numbers of animals per dose group (85/sex/group, with interim sacrifices of 10/sex/group at 6 and 12 months), and detailed reporting of methods and results (including individual animal data). The initial high dose (175 mg/kg-day) was reduced to 100 mg/kg-day at Week 11 due to high mortality.

When there is *suggestive evidence* of carcinogenicity to humans, EPA generally would not conduct a dose-response assessment and derive a cancer value. However, when the evidence includes a well-conducted study (as is the case with RDX), quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a).

An OSF was derived that considered the combination of female mouse liver and lung tumors. In modeling these data sets, the highest dose group was excluded because of the initial high mortality (loss of almost half the mice in that dose group). BMD and benchmark dose lower confidence limit (BMDL) estimates were calculated that correspond to a 10% extra risk (ER) of either tumor. The $BMDL_{10}$ so derived was extrapolated to the HED using body-weight scaling to the ¾ power ($BW^{3/4}$), and an OSF was derived by linear extrapolation from the $BMDL_{10}$ expressed as an HED ($BMDL_{10-HED}$). The OSF is 0.08 per mg/kg-day, based on the liver and lung tumor response in female mice (Lish et al., 1984).

ES.5. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

An inhalation unit risk (IUR) value was not calculated because inhalation carcinogenicity data for RDX are not available. While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support an

inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an IUR from the OSF. Thus, a quantitative cancer assessment was not conducted for inhalation exposure.

ES.6. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Life stage, particularly childhood, susceptibility has not been well-studied in human or animal studies of RDX toxicity. In rats, transfer of RDX from the dam to the fetus during gestation and to pups via maternal milk has been reported; however, reproductive and developmental toxicity studies did not identify effects in offspring at doses below those that also caused maternal toxicity. Yet, based on the primary mode of action for RDX exposure-induced nervous system effects (GABA receptor antagonism), and the fact that GABAergic signaling plays a prominent role in nervous system development, a significant concern is raised regarding the potential for developmental neurotoxicity. In addition, data on the incidence of convulsions and mortality provide some indication that pregnant animals may be a susceptible population, although the evidence is inconclusive. Data to suggest that males may be more susceptible than females to noncancer toxicity associated with RDX are limited. Some evidence suggests that cytochrome P450 (CYP450) enzymes may be involved in the metabolism of RDX, indicating a potential for genetic polymorphisms in these metabolic enzymes to affect susceptibility to RDX. Similarly, individuals with epilepsy or other seizure syndromes that have their basis in genetic mutation to $GABA_A$ receptors (GABA receptors that are ligand-gated ion channels, also known as ionotropic receptors) may represent another group that may be susceptible to RDX exposure; however, there is no information to indicate how genetic polymorphisms may affect susceptibility to RDX.

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