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

# (CASRN 121-82-4)

# In Support of Summary Information on the Integrated Risk Information System (IRIS)

September 2014

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# ABBREVIATIONS

| | |
|---|---|
| AAP | Army ammunition plants |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| AChE | acetylcholinesterase |
| ADAF | age-dependent adjustment factor |
| ALP | alkaline phosphatase |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| atm | atmosphere |
| ATSDR | Agency for Toxic Substances and Disease Registry |
| AUC | area under the curve |
| BDNF | brain-derived neurotrophic factor |
| BMC | benchmark concentration |
| BMCL | benchmark concentration lower confidence limit |
| BMD | benchmark dose |
| BMDL | benchmark dose lower confidence limit |
| BMDS | Benchmark Dose Software |
| BMR | benchmark response |
| BUN | blood urea nitrogen |
| BW | body weight |
| CASRN | Chemical Abstracts Service Registry Number |
| CCL | Contaminant Candidate List |
| CI | confidence interval |
| CNS | central nervous system |
| CYP450 | cytochrome P450 |
| DAF | dosimetric adjustment factor |
| DMSO | dimethylsulfoxide |
| DNA | deoxyribonucleic acid |
| DTIC | Defense Technical Information Center |
| EPA | Environmental Protection Agency |
| ER | extra risk |
| FDA | Food and Drug Administration |
| FOB | functional observational battery |
| GABA | gamma amino butyric acid |
| GD | gestational day |
| GLP | good laboratory practices |
| HEC | human equivalent concentration |
| HED | human equivalent dose |
| HERO | Health and Environmental Research Online |
| IARC | International Agency for Research on Cancer |
| IOM | Institute of Medicine |
| IRIS | Integrated Risk Information System |
| LDH | lactate dehydrogenase |
| LOAEL | lowest-observed-adverse-effect level |
| LOD | limit of detection |
| miRNA | microRNA |
| MOA | mode of action |
| NCEA | National Center for Environmental Assessment |
| NHANES | National Health and Nutrition Examination Survey |
| NICNAS | National Industrial Chemicals Notification and Assessment Scheme |
| NIOSH | National Institute for Occupational Safety and Health |
| NOAEL | no-observed-adverse-effect level |
| NPL | National Priorities List |
| NRC | Nuclear Regulatory Commission |
| NTP | National Toxicology Program |
| OR | odds ratio |
| ORD | Office of Research and Development |
| OSF | oral slope factor |
| OSHA | Occupational Safety and Health Administration |
| PBPK | physiologically based pharmacokinetic |
| POD | point of departure |
| $POD_{[ADJ]}$ | duration-adjusted POD |
| PWG | Pathology Working Group |
| RBC | red blood cell |
| RfC | inhalation reference concentration |
| RfD | oral reference dose |
| RNA | ribonucleic acid |
| SD | Sprague-Dawley |
| SDMS | spontaneous death or moribund sacrifice |
| SDWA | Safe Drinking Water Act |
| SGOT | glutamic oxaloacetic transaminase, also known as AST |
| SGPT | glutamic pyruvic transaminase, also known as ALT |
| SLE | systemic lupus erythematosus |
| SS | scheduled sacrifice |
| TNT | trinitrotoluene |
| TSCATS | Toxic Substances Control Act Test Submissions |
| TWA | time-weighted average |
| U.S. | United States of America |
| UCL | upper confidence limit |
| UCM | Unregulated Contaminant Monitoring |
| UF | uncertainty factor |
| $UF_A$ | animal-to-human uncertainty factor |
| $UF_D$ | database deficiencies uncertainty factor |
| $UF_H$ | human variation uncertainty factor |
| $UF_L$ | LOAEL-to-NOAEL uncertain factor |
| $UF_S$ | subchronic-to-chronic uncertainty factor |
| WBC | white blood cells |
| WOS | Web of Science |

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# PREFACE

This Toxicological Review critically reviews the publicly available studies on hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in order to identify its adverse health effects and to characterize exposure-response relationships. It was prepared under the auspices of EPA's Integrated Risk Information System (IRIS) program. This assessment updates a previous IRIS assessment of RDX that included an oral reference dose (RfD) for effects other than cancer (posted in 1988), a determination on the carcinogenicity of RDX, as well as derivation of an oral slope factor to quantify the cancer risk associated with RDX exposure (posted in 1990). New information has become available and this assessment reviews information on all health effects by all exposure routes. Organ/system-specific RfDs are calculated based on data for applicable hazards, e.g., nervous system toxicity. These reference values may be useful for cumulative risk assessments that consider the combined effect of multiple agents acting on the same biological system.

This assessment was conducted in accordance with EPA guidance, which is cited and summarized in the Preamble to IRIS Toxicological Reviews. The findings of this assessment and related documents produced during its development are available on the IRIS web site (http://www.epa.gov/iris). Appendices for assessments by other health agencies, chemical and physical properties, toxicokinetic information, and summaries of supporting toxicity information are provided as Supplemental Information to this assessment (See Appendices A to C).

A public meeting was held in December 2013 to obtain input on preliminary materials for RDX, including draft literature searches and associated search strategies, evidence tables, and exposure-response arrays prior to the development of the IRIS assessment. All public comments provided were taken into consideration in developing the draft assessment. The complete set of public comments are available on the docket at http://www.regulations.gov (Docket ID No. EPA-HQ-ORD-2013-0430).

In April 2011, the National Research Council (NRC) released its *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde*. In addition to offering comments specifically about EPA's draft formaldehyde assessment, the NRC made several recommendations to EPA for improving the development of IRIS assessments. EPA agreed with the recommendations and is implementing them consistent with the Panel's "Roadmap for Revision," which viewed the full implementation of their recommendations by the IRIS Program as a multi-year process.

In response to the NRC's 2011 recommendations, the IRIS Program has made changes to streamline the assessment development process, improve transparency, and create efficiencies in the Program. The NRC has acknowledged EPA's successes in this area. In May 2014, the NRC released their report *Review of EPA's Integrated Risk Information System Process* reviewing the IRIS

assessment development process and found that EPA has made substantial improvements to the IRIS Program in a short amount of time.

The draft RDX assessment represents a significant advancement in implementing the NRC recommendations. This assessment is streamlined, and uses tables, figures, and appendices to increase transparency and clarity. It is structured to have distinct sections for the literature search and screening strategy, study selection and evaluation, hazard identification, and dose-response assessment. The assessment includes a comprehensive, systematic, and documented literature search and screening approach, provides the database search strategy in a table (databases, keywords), visually represents the inclusion and exclusion of studies in a flow diagram, and all of the references are integrated within the Health and Environmental Research Online (HERO) database. A study evaluation section provides a systematic review of methodological aspects of epidemiology and experimental animal studies, including study design, conduct, and reporting, that was subsequently taken into consideration in the evaluation and synthesis of data from these studies. The evidence is presented in standardized evidence tables, and exposure-response arrays. The hazard identification and dose-response sections include subsections based on organ/system-specific effects in which the evidence is synthesized within and integrated across all evidence for each target organ/systems.

In the draft RDX assessment, the IRIS Program has attempted to transparently and uniformly identify strengths and limitations that would affect interpretation of results. All human and animal studies of RDX that were considered to be of acceptable quality, whether yielding positive, negative, or null results, were considered in assessing the evidence for health effects associated with chronic exposure to RDX. These studies were evaluated for aspects of design, conduct, and reporting that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of human hazard potential using the study quality considerations outlined in the Preamble. A brief summary of the evaluation is included in the section on methods for study selection and evaluation. Information on study features related to this evaluation is reported in evidence tables and documented in the synthesis of evidence. Discussion of study strengths and limitations (that ultimately supported preferences for the studies and data relied upon) were included in the text where relevant.

In this assessment, the IRIS Program is using existing guidelines to systematically approach the integration of noncancer human, animal, and mechanistic evidence. In conducting this analysis and developing the synthesis, the IRIS Program evaluates the data for the: strength of the relationship between the exposure and response and the presence of a dose-response relationship; specificity of the response to chemical exposure and whether the exposure precedes the effect; consistency of the association between the chemical exposure and response; and biological plausibility of the response or effect and its relevance to humans. The IRIS Program uses this weight-of-evidence approach to identify the potential human hazards associated with chemical exposure.

The IRIS RDX assessment provides a streamlined presentation of information, integrated hazard identification of all toxic effects, and derivation of organ/system-specific reference values. Additionally, consistent with the goal that assessments should provide a scientifically sound and transparent evaluation of the relevant scientific literature and presentation of the analyses performed, this assessment contains an expanded discussion of study selection and evaluation, as well as increased documentation of key assessment decisions.

For additional information about this assessment or for general questions regarding IRIS, please contact EPA's IRIS Hotline at 202-566-1676 (phone), 202-566-1749 (fax), or hotline.iris@epa.gov.

**Chemical Properties**

RDX is a white, crystalline solid member of the nitramine class of organic nitrate explosives (Boileau et al., 2005; Bingham et al., 2001). It is a synthetic chemical not found naturally in the environment. The solubility of RDX in water is poor, having been reported as 59.7 mg/L at 25°C (Yalkowsky and He, 2003). The Henry's law constant for RDX is approximately $2 \times 10^{-11}$ atm-$m^3$/mole at 25°C, suggesting slow volatilization from water or moist soil (ATSDR, 2012). The normalized soil organic carbon/water partition coefficient ($K_{OC}$) values for RDX range from 42 to 167, indicating a potential for RDX to be mobile in soil (Spanggord et al., 1980). The vapor pressure of $4.10 \times 10^{-9}$ mm Hg at 20°C suggests that it will exist as particulate matter in air and be removed by both wet and dry deposition. RDX degrades in the environment, and can be subject to both photolysis (Sikka et al., 1980; Spanggord et al., 1980) and biodegradation (Funk et al., 1993; Mccormick et al., 1981). Further information on the physical and chemical properties of RDX are provided in Appendix C, Section C.1.

**Uses and Environmental Occurrence**

RDX is used primarily as a military explosive. In the United States, RDX is produced at Army ammunition plants (AAP) and is not produced commercially. RDX production peaked in the 1960s; 180 million pounds per year were produced from 1969 to 1971. Yearly total production dropped to 16 million pounds in 1984 (ATSDR, 2012). According to the U.S. EPA Inventory Update Reporting program, the aggregated national production volume in 2006 was between 1 and 10 million pounds.

RDX releases have been reported into the air, water, or soil (ATSDR, 2012, 1999, 1993, 1992). RDX is mobile in soil; leaching into groundwater has been reported in samples from military facilities (Best et al., 1999a; Godejohann et al., 1998; Bart et al., 1997; Steuckart et al., 1994; Spanggord et al., 1980). RDX transport in soil is generally through dissolution by precipitation and subsequent downward movement, including migration to groundwater aquifers, and not much via surface runoff (U.S. EPA, 2012b). An extensive discussion of RDX properties and fate and transport is available in U.S. EPA (2012b). Detectable levels of RDX have been observed in plants irrigated or grown with RDX-contaminated water (Best et al., 1999b; Simini and Checkai, 1996; Harvey et al.,

1991). RDX has also been detected in indoor air samples from military facilities where RDX is produced (Bishop et al., 1988).

Exposures to RDX among the general population are likely to be confined to individuals in or around military facilities where RDX is or was produced, stored, or used. Oral, inhalation, and dermal routes of exposure may be relevant.

RDX has been detected in surface water, groundwater, sediment, or soil at 34 current U.S. EPA National Priorities List (NPL) sites. The NPL serves as a list of sites with known releases or threatened releases of hazardous substances, pollutants, or contaminants throughout the United States and its territories. The NPL list aids the Agency in identifying the most serious sites that may warrant cleanup. The majority of the NPL sites where RDX was listed are associated with military facilities, although the total number of sites where RDX is present is unknown.

RDX is not regulated under the Safe Drinking Water Act (SDWA), although it was included as a contaminant to be monitored under the Unregulated Contaminant Monitoring (UCM) Rule by EPA's Office of Water from 2007 to 2011. Contaminants included in the UCM program are suspected of being present in drinking water, but do not have existing health-based standards set under the SDWA. RDX has also been included the Office of Water's Drinking Water Contaminant Candidate Lists (CCL) since the initial listing was published in 1998. The presence of a chemical on the list suggests that it is known or anticipated to occur in public water systems.

**Assessments by Other National and International Health Agencies**

Toxicity values for RDX have been established by the Agency for Toxic Substances and Disease Registry (ATSDR), the American Conference of Governmental Industrial Hygienists (ACGIH), the Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS), the National Institute for Occupational Safety and Health (NIOSH), and the Occupational Safety and Health Administration (OSHA). These toxicity values and their basis are presented in Appendix A. It is important to recognize that the assessments performed by other health agencies may have been prepared for different purposes and may utilize different methods, and that newer studies may be included in the IRIS assessment.

# PREAMBLE TO IRIS TOXICOLOGICAL REVIEWS

## 1. Scope of the IRIS Program

Soon after the EPA was established in 1970, it was at the forefront of developing risk assessment as a science and applying it in decisions to protect human health and the environment. The Clean Air Act, for example, mandates that the EPA provide "an ample margin of safety to protect public health"; the Safe Drinking Water Act, that "no adverse effects on the health of persons may reasonably be anticipated to occur, allowing an adequate margin of safety." Accordingly, the EPA uses information on the adverse effects of chemicals and on exposure levels below which these effects are not anticipated to occur.

IRIS assessments critically review the publicly available studies to identify adverse health effects from exposure to chemicals and to characterize exposure-response relationships. In terms set forth by the National Research Council (NRC, 1983), IRIS assessments cover the hazard identification and dose-response assessment steps of risk assessment, not the exposure assessment or risk characterization steps that are conducted by the EPA's program and regional offices and by other federal, state, and local health agencies that evaluate risk in specific populations and exposure scenarios. IRIS assessments are distinct from and do not address political, economic, and technical considerations that influence the design and selection of risk management alternatives.

An IRIS assessment may cover a single chemical, a group of structurally or toxicologically related chemicals, or a complex mixture. These agents may be found in air, water, soil, or sediment. Exceptions are chemicals currently used exclusively as pesticides, ionizing and non-ionizing radiation, and criteria air pollutants listed under Section 108 of the Clean Air Act (carbon monoxide, lead, nitrogen oxides, ozone, particulate matter, and sulfur oxides).

Periodically, the IRIS Program asks other EPA programs and regions, other federal agencies, state health agencies, and the general public to nominate chemicals and mixtures for future assessment or reassessment. Agents may be considered for reassessment as significant new studies are published. Selection is based on program and regional office priorities and on availability of adequate information to evaluate the potential for adverse effects. Other agents may also be assessed in response to an urgent public health need.

## 2. Process for developing and peer-reviewing IRIS assessments

The process for developing IRIS assessments (revised in May 2009 and enhanced in July 2013) involves critical analysis of the pertinent studies, opportunities for public input, and multiple levels of scientific review. The EPA revises draft assessments after each review, and external drafts and comments become part of the public record (U.S. EPA, 2009).

Before beginning an assessment, the IRIS Program discusses the scope with other EPA programs and regions to ensure that the assessment will meet their needs. Then a public meeting on problem formulation invites discussion of the key issues and the studies and analytical approaches that might contribute to their resolution.

**Step 1. Development of a draft Toxicological Review**. The draft assessment considers all pertinent publicly available studies and applies

consistent criteria to evaluate study quality, identify health effects, identify mechanistic events and pathways, integrate the evidence of causation for each effect, and derive toxicity values. A public meeting prior to the integration of evidence and derivation of toxicity values promotes public discussion of the literature search, evidence, and key issues.

**Step 2. Internal review by scientists in EPA programs and regions**. The draft assessment is revised to address the comments from within the EPA.

**Step 3. Interagency science consultation with other federal agencies and the Executive Offices of the President.** The draft assessment is revised to address the interagency comments. The science consultation draft, interagency comments, and the EPA's response to major comments become part of the public record.

**Step 4. Public review and comment, followed by external peer review**. The EPA releases the draft assessment for public review and comment. A public meeting provides an opportunity to discuss the assessment prior to peer review. Then the EPA releases a draft for external peer review. The peer review meeting is open to the public and includes time for oral public comments. The peer reviewers assess whether the evidence has been assembled and evaluated according to guidelines and whether the conclusions are justified by the evidence. The peer review draft, written public comments, and peer review report become part of the public record.

**Step 5. Revision of draft Toxicological Review and development of draft IRIS summary**. The draft assessment is revised to reflect the peer review comments, public comments, and newly published studies that are critical to the conclusions of the assessment. The disposition of peer review comments and public comments becomes part of the public record.

**Step 6. Final EPA review and interagency science discussion with other federal agencies and the Executive Offices of the President** The draft assessment and summary are revised to address the EPA and interagency comments. The science discussion draft, written interagency comments, and EPA's response to major comments become part of the public record.

**Step 7. Completion and posting**. The Toxicological Review and IRIS summary are posted on the IRIS website (http://www.epa.gov/iris/).

The remainder of this Preamble addresses step 1, the development of a draft Toxicological Review. IRIS assessments follow standard practices of evidence evaluation and peer review, many of which are discussed in EPA guidelines (U.S. EPA, 2005a, b, 2000b, 1998, 1996, 1991, 1986a, b) and other methods (U.S. EPA, 2012a, b, 2011, 2006a, b, 2002, 1994). Transparent application of scientific judgment is of paramount importance. To provide a harmonized approach across IRIS assessments, this Preamble summarizes concepts from these guidelines and emphasizes principles of general applicability.

# 3. Identifying and selecting pertinent studies

## 3.1. Identifying studies

Before beginning an assessment, the EPA conducts a comprehensive search of the primary scientific literature. The literature search follows standard practices and includes the PubMed and ToxNet databases of the National Library of Medicine, Web of Science, and other databases listed in the EPA's HERO system (Health and Environmental Research Online, http://hero.epa.gov/). Searches for information on mechanisms of toxicity are inherently specialized and may include studies on other agents that act through related mechanisms.

Each assessment specifies the search strategies, keywords, and cut-off dates of its literature searches. The EPA posts the results of the literature search on the IRIS web site and requests information from the public on additional studies and ongoing research.

The EPA also considers studies received through the IRIS Submission Desk and studies (typically unpublished) submitted under the Toxic Substances Control Act or the Federal Insecticide, Fungicide, and Rodenticide Act. Material submitted as Confidential Business Information is considered only if it includes health and safety data that can be publicly released. If a study that may be critical to the conclusions of the assessment has not been peer-reviewed, the EPA will have it peer-reviewed.

The EPA also examines the toxicokinetics of the agent to identify other chemicals (for example, major metabolites of the agent) to include in the assessment if adequate information is available, in order to more fully explain the toxicity of the agent and to suggest dose metrics for subsequent modeling.

In assessments of chemical mixtures, mixture studies are preferred for their ability to reflect interactions among components.

The literature search seeks, in decreasing order of preference (U.S. EPA, 2000b, §2.2; 1986b, §2.1)]:

- Studies of the mixture being assessed.
- Studies of a sufficiently similar mixture. In evaluating similarity, the assessment considers the alteration of mixtures in the environment through partitioning and transformation.
- Studies of individual chemical components of the mixture, if there are not adequate studies of sufficiently similar mixtures.

## 3.2. Selecting pertinent epidemiologic studies

Study design is the key consideration for selecting pertinent epidemiologic studies from the results of the literature search.

- Cohort studies, case-control studies, and some population-based surveys (for example, NHANES) provide the strongest epidemiologic evidence, especially if they collect information about individual exposures and effects.
- Ecological studies (geographic correlation studies) relate exposures and effects by geographic area. They can provide strong evidence if there are large exposure contrasts between geographic areas, relatively little exposure variation within study areas, and population migration is limited.
- Case reports of high or accidental exposure lack definition of the population at risk and the expected number of cases. They can provide information about a rare effect or about the relevance of analogous results in animals.

The assessment briefly reviews ecological studies and case reports but reports details only if they suggest effects not identified by other studies.

### 3.3. Selecting pertinent experimental studies

Exposure route is a key design consideration for selecting pertinent experimental animal studies or human clinical studies.

- Studies of oral, inhalation, or dermal exposure involve passage through an absorption barrier and are considered most pertinent to human environmental exposure.
- Injection or implantation studies are often considered less pertinent but may provide valuable toxicokinetic or mechanistic information. They also may be useful for identifying effects in animals if deposition or absorption is problematic (for example, for particles and fibers).

Exposure duration is also a key design consideration for selecting pertinent experimental animal studies.

- Studies of effects from chronic exposure are most pertinent to lifetime human exposure.
- Studies of effects from less-than-chronic exposure are pertinent but less preferred for identifying effects from lifetime human exposure. Such studies may be indicative of effects from less-than-lifetime human exposure.

Short-duration studies involving animals or humans may provide toxicokinetic or mechanistic information.

For developmental toxicity and reproductive toxicity, irreversible effects may result from a brief exposure during a critical period of development. Accordingly, specialized study designs are used for these effects (U.S. EPA, 2006b, 1998, 1996, 1991).

## 4. Evaluating the quality of individual studies

After the subsets of pertinent epidemiologic and experimental studies have been selected from the literature searches, the assessment evaluates the quality of each individual study. This evaluation considers the design, methods, conduct, and documentation of each study, but not whether the results are positive, negative, or null. The objective is to identify the stronger, more informative studies based on a uniform evaluation of quality characteristics across studies of similar design.

### 4.1. Evaluating the quality of epidemiologic studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each epidemiologic study in the overall evaluation (U.S. EPA, 2005a, 1998, 1996, 1994, 1991):

- Documentation of study design, methods, population characteristics, and results.
- Definition and selection of the study group and comparison group.
- Ascertainment of exposure to the chemical or mixture.
- Ascertainment of disease or health effect.
- Duration of exposure and follow-up and adequacy for assessing the occurrence of effects.
- Characterization of exposure during critical periods.
- Sample size and statistical power to detect anticipated effects.
- Participation rates and potential for selection bias as a result of the achieved participation rates.
- Measurement error (can lead to misclassification of exposure, health

outcomes, and other factors) and other types of information bias.

- Potential confounding and other sources of bias addressed in the study design or in the analysis of results. The basis for consideration of confounding is a reasonable expectation that the confounder is related to both exposure and outcome and is sufficiently prevalent to result in bias.

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating epidemiologic studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991).

## 4.2. Evaluating the quality of experimental studies

The assessment evaluates design and methodological aspects that can increase or decrease the weight given to each experimental animal study, in-vitro study, or human clinical study (U.S. EPA, 2005a, 1998, 1996, 1991). Research involving human subjects is considered only if conducted according to ethical principles.

- Documentation of study design, animals or study population, methods, basic data, and results.
- Nature of the assay and validity for its intended purpose.
- Characterization of the nature and extent of impurities and contaminants of the administered chemical or mixture.
- Characterization of dose and dosing regimen (including age at exposure) and their adequacy to elicit adverse effects, including latent effects.
- Sample sizes and statistical power to detect dose-related differences or trends.
- Ascertainment of survival, vital signs, disease or effects, and cause of death.
- Control of other variables that could influence the occurrence of effects.

The assessment uses statistical tests to evaluate whether the observations may be due to chance. The standard for determining statistical significance of a response is a trend test or comparison of outcomes in the exposed groups against those of concurrent controls. In some situations, examination of historical control data from the same laboratory within a few years of the study may improve the analysis. For an uncommon effect that is not statistically significant compared with concurrent controls, historical controls may show that the effect is unlikely to be due to chance. For a response that appears significant against a concurrent control response that is unusual, historical controls may offer a different interpretation (U.S. EPA, 2005a, §2.2.2.1.3).

For developmental toxicity, reproductive toxicity, neurotoxicity, and cancer there is further guidance on the nuances of evaluating experimental studies of these effects (U.S. EPA, 2005a, 1998, 1996, 1991). In multi-generation studies, agents that produce developmental effects at doses that are not toxic to the maternal animal are of special concern. Effects that occur at doses associated with mild maternal toxicity are not assumed to result only from maternal toxicity. Moreover, maternal effects may be reversible, while effects on the offspring may be permanent (U.S. EPA, 1998, §3.1.2.4.5.4; 1991, §3.1.1.4),.

## 4.3. Reporting study results

The assessment uses evidence tables to present the design and key results of pertinent studies. There may be separate tables for each site of toxicity or type of study.

If a large number of studies observe the same effect, the assessment considers the study quality characteristics in this section to identify the strongest studies or types of study. The tables present details from these studies, and the assessment explains the reasons for not reporting details of other studies or groups of studies that do not add new information. Supplemental information

provides references to all studies considered, including those not summarized in the tables.

The assessment discusses strengths and limitations that affect the interpretation of each study. If the interpretation of a study in the assessment differs from that of the study authors, the assessment discusses the basis for the difference.

As a check on the selection and evaluation of pertinent studies, the EPA asks peer reviewers to identify studies that were not adequately considered.

# 5. Evaluating the overall evidence of each effect

## 5.1. Concepts of causal inference

For each health effect, the assessment evaluates the evidence as a whole to determine whether it is reasonable to infer a causal association between exposure to the agent and the occurrence of the effect. This inference is based on information from pertinent human studies, animal studies, and mechanistic studies of adequate quality. Positive, negative, and null results are given weight according to study quality.

Causal inference involves scientific judgment, and the considerations are nuanced and complex. Several health agencies have developed frameworks for causal inference, among them the U.S. Surgeon General (CDC, 2004; HEW, 1964), the International Agency for Research on Cancer (IARC, 2006), the Institute of Medicine (IOM, 2008), and the EPA (2010, §1.6; 2005a, §2.5). Although developed for different purposes, the frameworks are similar in nature and provide an established structure and language for causal inference. Each considers aspects of an association that suggest causation, discussed by Hill (1965) and elaborated by Rothman and Greenland (1998), and U.S. EPA (2005a, §2.2.1.7; 1994, Appendix C).

**Strength of association:** The finding of a large relative risk with narrow confidence intervals strongly suggests that an association is not due to chance, bias, or other factors. Modest relative risks, however, may reflect a small range of exposures, an agent of low potency, an increase in an effect that is common, exposure misclassification, or other sources of bias.

**Consistency of association:** An inference of causation is strengthened if elevated risks are observed in independent studies of different populations and exposure scenarios. Reproducibility of findings constitutes one of the strongest arguments for causation. Discordant results sometimes reflect differences in study design, exposure, or confounding factors.

**Specificity of association:** As originally intended, this refers to one cause associated with one effect. Current understanding that many agents cause multiple effects and many effects have multiple causes make this a less informative aspect of causation, unless the effect is rare or unlikely to have multiple causes.

**Temporal relationship:** A causal interpretation requires that exposure precede development of the effect.

**Biologic gradient (exposure-response relationship):** Exposure-response relationships strongly suggest causation. A monotonic increase is not the only pattern consistent with causation. The presence of an exposure-response gradient also weighs against bias and confounding as the source of an association.

**Biologic plausibility:** An inference of causation is strengthened by data demonstrating plausible biologic mechanisms, if available. Plausibility may reflect subjective prior beliefs if there is insufficient understanding of the biologic process involved.

**Coherence:** An inference of causation is strengthened by supportive results from animal experiments, toxicokinetic studies, and short-term tests. Coherence may also

be found in other lines of evidence, such as changing disease patterns in the population.

**"Natural experiments":** A change in exposure that brings about a change in disease frequency provides strong evidence, as it tests the hypothesis of causation. An example would be an intervention to reduce exposure in the workplace or environment that is followed by a reduction of an adverse effect.

**Analogy:** Information on structural analogues or on chemicals that induce similar mechanistic events can provide insight into causation.

These considerations are consistent with guidelines for systematic reviews that evaluate the quality and weight of evidence. Confidence is increased if the magnitude of effect is large, if there is evidence of an exposure-response relationship, or if an association was observed and the plausible biases would tend to decrease the magnitude of the reported effect. Confidence is decreased for study limitations, inconsistency of results, indirectness of evidence, imprecision, or reporting bias (Guyatt et al., 2008b; Guyatt et al., 2008a).

## 5.2. Evaluating evidence in humans

For each effect, the assessment evaluates the evidence from the epidemiologic studies as a whole. The objective is to determine whether a credible association has been observed and, if so, whether that association is consistent with causation. In doing this, the assessment explores alternative explanations (such as chance, bias, and confounding) and draws a conclusion about whether these alternatives can satisfactorily explain any observed association.

To make clear how much the epidemiologic evidence contributes to the overall weight of the evidence, the assessment may select a standard descriptor to characterize the epidemiologic evidence of association between exposure to the agent and occurrence of a health effect.

***Sufficient epidemiologic evidence of an association consistent with causation:*** The evidence establishes a causal association for which alternative explanations such as chance, bias, and confounding can be ruled out with reasonable confidence.

***Suggestive epidemiologic evidence of an association consistent with causation:*** The evidence suggests a causal association but chance, bias, or confounding cannot be ruled out as explaining the association.

***Inadequate epidemiologic evidence to infer a causal association:*** The available studies do not permit a conclusion regarding the presence or absence of an association.

***Epidemiologic evidence consistent with no causal association:*** Several adequate studies covering the full range of human exposures and considering susceptible populations, and for which alternative explanations such as bias and confounding can be ruled out, are mutually consistent in not finding an association.

## 5.3. Evaluating evidence in animals

For each effect, the assessment evaluates the evidence from the animal experiments as a whole to determine the extent to which they indicate a potential for effects in humans. Consistent results across various species and strains increase confidence that similar results would occur in humans. Several concepts discussed by Hill (1965) are pertinent to the weight of experimental results: consistency of response, dose-response relationships, strength of response, biologic plausibility, and coherence (U.S. EPA, 2005a, §2.2.1.7; 1994, Appendix C).

In weighing evidence from multiple experiments, U.S. EPA (2005a, §2.5) distinguishes:

***Conflicting evidence*** (that is, mixed positive and negative results in the same sex and strain using a similar study protocol) from

***Differing results*** (that is, positive results and negative results are in different sexes or strains or use different study protocols).

Negative or null results do not invalidate positive results in a different experimental system. The EPA regards all as valid observations and looks to explain differing results using mechanistic information (for example, physiologic or metabolic differences across test systems) or methodological differences (for example, relative sensitivity of the tests, differences in dose levels, insufficient sample size, or timing of dosing or data collection).

It is well established that there are critical periods for some developmental and reproductive effects (U.S. EPA, 2006b, 2005a, b, 1998, 1996, 1991). Accordingly, the assessment determines whether critical periods have been adequately investigated. Similarly, the assessment determines whether the database is adequate to evaluate other critical sites and effects.

In evaluating evidence of genetic toxicity:

- Demonstration of gene mutations, chromosome aberrations, or aneuploidy in humans or experimental mammals (*in vivo*) provides the strongest evidence.
- This is followed by positive results in lower organisms or in cultured cells (*in vitro*) or for other genetic events.
- Negative results carry less weight, partly because they cannot exclude the possibility of effects in other tissues (IARC, 2006).

For germ-cell mutagenicity, The EPA has defined categories of evidence, ranging from positive results of human germ-cell mutagenicity to negative results for all effects of concern (U.S. EPA, 1986a, §2.3).

## 5.4. Evaluating mechanistic data

Mechanistic data can be useful in answering several questions.

- The biologic plausibility of a causal interpretation of human studies.
- The generalizability of animal studies to humans.
- The susceptibility of particular populations or lifestages.

The focus of the analysis is to describe, if possible, mechanistic pathways that lead to a health effect. These pathways encompass:

- *Toxicokinetic processes* of absorption, distribution, metabolism, and elimination that lead to the formation of an active agent and its presence at the site of initial biologic interaction.
- *Toxicodynamic processes* that lead to a health effect at this or another site (also known as a *mode of action*).

For each effect, the assessment discusses the available information on its *modes of action* and associated *key events* (*key events* being empirically observable, necessary precursor steps or biologic markers of such steps; *mode of action* being a series of key events involving interaction with cells, operational and anatomic changes, and resulting in disease). Pertinent information may also come from studies of metabolites or of compounds that are structurally similar or that act through similar mechanisms. Information on mode of action is not required for a conclusion that the agent is causally related to an effect (U.S. EPA, 2005a, §2.5).

The assessment addresses several questions about each hypothesized mode of action (U.S. EPA, 2005a, §2.4.3.4).

1) **Is the hypothesized mode of action sufficiently supported in test animals?** Strong support for a key event being necessary to a mode of action can come from experimental challenge to the hypothesized mode of action, in which studies that suppress a key event observe suppression of the effect. Support for a mode of action is meaningfully strengthened by consistent results in different experimental models, much

more so than by replicate experiments in the same model. The assessment may consider various aspects of causation in addressing this question.

2) **Is the hypothesized mode of action relevant to humans?** The assessment reviews the key events to identify critical similarities and differences between the test animals and humans. Site concordance is not assumed between animals and humans, though it may hold for certain effects or modes of action. Information suggesting quantitative differences in doses where effects would occur in animals or humans is considered in the dose-response analysis. Current levels of human exposure are not used to rule out human relevance, as IRIS assessments may be used in evaluating new or unforeseen circumstances that may entail higher exposures.

3) **Which populations or lifestages can be particularly susceptible to the hypothesized mode of action?** The assessment reviews the key events to identify populations and lifestages that might be susceptible to their occurrence. Quantitative differences may result in separate toxicity values for susceptible populations or lifestages.

The assessment discusses the likelihood that an agent operates through multiple modes of action. An uneven level of support for different modes of action can reflect disproportionate resources spent investigating them (U.S. EPA, 2005a, §2.4.3.3). It should be noted that in clinical reviews, the credibility of a series of studies is reduced if evidence is limited to studies funded by one interested sector (Guyatt et al., 2008a).

For cancer, the assessment evaluates evidence of a mutagenic mode of action to guide extrapolation to lower doses and consideration of susceptible lifestages. Key data include the ability of the agent or a metabolite to react with or bind to DNA, positive results in multiple test systems, or similar properties and structure-activity relationships to mutagenic carcinogens (U.S. EPA, 2005a ,§2.3.5).

## 5.5. Characterizing the overall weight of the evidence

After evaluating the human, animal, and mechanistic evidence pertinent to an effect, the assessment answers the question: Does the agent cause the adverse effect? (NRC, 2009, 1983). In doing this, the assessment develops a narrative that integrates the evidence pertinent to causation. To provide clarity and consistency, the narrative includes a standard hazard descriptor. For example, the following standard descriptors combine epidemiologic, experimental, and mechanistic evidence of carcinogenicity (U.S. EPA, 2005a, §2.5).

***Carcinogenic to humans:*** There is convincing epidemiologic evidence of a causal association (that is, there is reasonable confidence that the association cannot be fully explained by chance, bias, or confounding); or there is strong human evidence of cancer or its precursors, extensive animal evidence, identification of key precursor events in animals, and strong evidence that they are anticipated to occur in humans.

***Likely to be carcinogenic to humans:*** The evidence demonstrates a potential hazard to humans but does not meet the criteria for *carcinogenic*. There may be a plausible association in humans, multiple positive results in animals, or a combination of human, animal, or other experimental evidence.

***Suggestive evidence of carcinogenic potential:*** The evidence raises concern for effects in humans but is not sufficient for a stronger conclusion. This descriptor covers a range of evidence, from a positive result in the only available study to a single positive result in an extensive database that includes negative results in other species.

***Inadequate information to assess carcinogenic potential:*** No other descriptors apply. *Conflicting evidence* can be classified as *inadequate information* if all positive results are opposed by negative studies of equal quality in the same sex and strain. *Differing results*, however, can be classified as *suggestive evidence* or as *likely to be carcinogenic*.

***Not likely to be carcinogenic to humans:*** There is robust evidence for concluding that there is no basis for concern. There may be no effects in both sexes of at least two appropriate animal species; positive animal results and strong, consistent evidence that each mode of action in animals does not operate in humans; or convincing evidence that effects are not likely by a particular exposure route or below a defined dose.

Multiple descriptors may be used if there is evidence that carcinogenic effects differ by dose range or exposure route (U.S. EPA, 2005a, §2.5).

Another example of standard descriptors comes from the EPA's Integrated Science Assessments, which evaluate causation for the effects of the criteria pollutants in ambient air (U.S. EPA, 2010, §1.6).

***Causal relationship:*** Sufficient evidence to conclude that there is a causal relationship. Observational studies cannot be explained by plausible alternatives, or they are supported by other lines of evidence, for example, animal studies or mechanistic information.

***Likely to be a causal relationship:*** Sufficient evidence that a causal relationship is likely, but important uncertainties remain. For example, observational studies show an association but co-exposures are difficult to address or other lines of evidence are limited or inconsistent; or multiple animal studies from different laboratories demonstrate effects and there are limited or no human data.

***Suggestive of a causal relationship:*** At least one high-quality epidemiologic study shows an association but other studies are inconsistent.

***Inadequate to infer a causal relationship:*** The studies do not permit a conclusion regarding the presence or absence of an association.

***Not likely to be a causal relationship:*** Several adequate studies, covering the full range of human exposure and considering susceptible populations, are mutually consistent in not showing an effect at any level of exposure.

The EPA is investigating and may on a trial basis use these or other standard descriptors to characterize the overall weight of the evidence for effects other than cancer.

## 6. Selecting studies for derivation of toxicity values

For each effect where there is credible evidence of an association with the agent, the assessment derives toxicity values if there are suitable epidemiologic or experimental data. The decision to derive toxicity values may be linked to the hazard descriptor.

Dose-response analysis requires quantitative measures of dose and response. Then, other factors being equal:

- Epidemiologic studies are preferred over animal studies, if quantitative measures of exposure are available and effects can be attributed to the agent.
- Among experimental animal models, those that respond most like humans are preferred, if the comparability of response can be determined.
- Studies by a route of human environmental exposure are preferred, although a validated toxicokinetic model can be used to extrapolate across exposure routes.

- Studies of longer exposure duration and follow-up are preferred, to minimize uncertainty about whether effects are representative of lifetime exposure.
- Studies with multiple exposure levels are preferred for their ability to provide information about the shape of the exposure-response curve.
- Studies with adequate power to detect effects at lower exposure levels are preferred, to minimize the extent of extrapolation to levels found in the environment.

Studies with non-monotonic exposure-response relationships are not necessarily excluded from the analysis. A diminished effect at higher exposure levels may be satisfactorily explained by factors such as competing toxicity, saturation of absorption or metabolism, exposure misclassification, or selection bias.

If a large number of studies are suitable for dose-response analysis, the assessment considers the study characteristics in this section to focus on the most informative data. The assessment explains the reasons for not analyzing other groups of studies. As a check on the selection of studies for dose-response analysis, the EPA asks peer reviewers to identify studies that were not adequately considered.

# 7. Deriving toxicity values

## 7.1. General framework for dose-response analysis

The EPA uses a two-step approach that distinguishes analysis of the observed dose-response data from inferences about lower doses (U.S. EPA, 2005a, §3).

Within the observed range, the preferred approach is to use modeling to incorporate a wide range of data into the analysis. The modeling yields a *point of departure* (an exposure level near the lower end of the observed range, without significant extrapolation to lower doses) (Sections 7.2-7.3).

Extrapolation to lower doses considers what is known about the modes of action for each effect (Sections 7.4-7.5). If response estimates at lower doses are not required, an alternative is to derive *reference values*, which are calculated by applying factors to the point of departure in order to account for sources of uncertainty and variability (Section 7.6).

For a group of agents that induce an effect through a common mode of action, the dose-response analysis may derive a *relative potency factor* for each agent. A full dose-response analysis is conducted for one well-studied *index chemical* in the group, then the potencies of other members are expressed in relative terms based on relative toxic effects, relative absorption or metabolic rates, quantitative structure-activity relationships, or receptor binding characteristics (U.S. EPA, 2005a, §3.2.6; 2000b, §4.4).

Increasingly, the EPA is basing toxicity values on combined analyses of multiple data sets or multiple responses. The EPA also considers multiple dose-response approaches if they can be supported by robust data.

## 7.2. Modeling dose to sites of biologic effects

The preferred approach for analysis of dose is toxicokinetic modeling because of its ability to incorporate a wide range of data. The preferred dose metric would refer to the active agent at the site of its biologic effect or to a close, reliable surrogate measure. The active agent may be the administered chemical or a metabolite. Confidence in the use of a toxicokinetic model depends on the robustness of its validation process and on the results of sensitivity analyses (U.S. EPA, 2006a; 2005a, §3.1; 1994, §4.3).

Because toxicokinetic modeling can require many parameters and more data than are typically available, the EPA has developed standard approaches that can be applied to typical data sets. These standard approaches also facilitate comparison across exposure patterns and species.

- Intermittent study exposures are standardized to a daily average over the duration of exposure. For chronic effects, daily exposures are averaged over the lifespan. Exposures during a critical period, however, are not averaged over a longer duration (U.S. EPA, 2005a, §3.1.1; 1991, §3.2).

- Doses are standardized to equivalent human terms to facilitate comparison of results from different species.

- Oral doses are scaled allometrically using $mg/kg^{3/4}$-day as the equivalent dose metric across species. Allometric scaling pertains to equivalence across species, not across lifestages, and is not used to scale doses from adult humans or mature animals to infants or children (U.S. EPA, 2011; 2005a, §3.1.3).

- Inhalation exposures are scaled using dosimetry models that apply species-specific physiologic and anatomic factors and consider whether the effect occurs at the site of first contact or after systemic circulation (U.S. EPA, 2012a; 1994, §3).

It can be informative to convert doses across exposure routes. If this is done, the assessment describes the underlying data, algorithms, and assumptions (U.S. EPA, 2005a, §3.1.4).

In the absence of study-specific data on, for example, intake rates or body weight, the EPA has developed recommended values for use in dose-response analysis (U.S. EPA, 1988).

## 7.3. Modeling response in the range of observation

Toxicodynamic ("biologically based") modeling can incorporate data on biologic processes leading to an effect. Such models require sufficient data to ascertain a mode of action and to quantitatively support model parameters associated with its key events. Because different models may provide equivalent fits to the observed data but diverge substantially at lower doses, critical biologic parameters should be measured from laboratory studies, not by model fitting. Confidence in the use of a toxicodynamic model depends on the robustness of its validation process and on the results of sensitivity analyses. Peer review of the scientific basis and performance of a model is essential (U.S. EPA, 2005a, §3.2.2).

Because toxicodynamic modeling can require many parameters and more knowledge and data than are typically available, the EPA has developed a standard set of empirical ("curve-fitting") models (http://www.epa.gov/ncea/bmds/) that can be applied to typical data sets, including those that are nonlinear. The EPA has also developed guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results (U.S. EPA, 2012a). Additional judgment or alternative analyses are used if the procedure fails to yield reliable results, for example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses (U.S. EPA, 2005a, §3.2.3).

Modeling is used to derive a point of departure (U.S. EPA, 2012a; 2005a, §3.2.4). (See Section 7.6 for alternatives if a point of departure cannot be derived by modeling.):

- If linear extrapolation is used, selection of a response level corresponding to the point of departure is not highly influential, so standard values near the low end of the observable range are generally used (for example, 10% extra risk for cancer bioassay data, 1% for epidemiologic data, lower for rare cancers).

- For nonlinear approaches, both statistical and biologic considerations are taken into account.

- For dichotomous data, a response level of 10% extra risk is generally used for

minimally adverse effects, 5% or lower for more severe effects.

- For continuous data, a response level is ideally based on an established definition of biologic significance. In the absence of such definition, one control standard deviation from the control mean is often used for minimally adverse effects, one-half standard deviation for more severe effects.

The point of departure is the 95% lower bound on the dose associated with the selected response level.

## 7.4. Extrapolating to lower doses and response levels

The purpose of extrapolating to lower doses is to estimate responses at exposures below the observed data. Low-dose extrapolation, typically used for cancer data, considers what is known about modes of action (U.S. EPA, 2005a, §3.3.1 and §3.3.2).

1) If a biologically based model has been developed and validated for the agent, extrapolation may use the fitted model below the observed range if significant model uncertainty can be ruled out with reasonable confidence.

2) Linear extrapolation is used if the dose-response curve is expected to have a linear component below the point of departure. This includes:

   - Agents or their metabolites that are DNA-reactive and have direct mutagenic activity.
   - Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect.

   Linear extrapolation is also used when data are insufficient to establish mode of action and when scientifically plausible.

   The result of linear extrapolation is described by an oral slope factor or an inhalation unit risk, which is the slope of the dose-response curve at lower doses or concentrations, respectively.

3) Nonlinear models are used for extrapolation if there are sufficient data to ascertain the mode of action and to conclude that it is not linear at lower doses, and the agent does not demonstrate mutagenic or other activity consistent with linearity at lower doses. Nonlinear approaches generally should not be used in cases where mode of action has not ascertained. If nonlinear extrapolation is appropriate but no model is developed, an alternative is to calculate reference values.

4) Both linear and nonlinear approaches may be used if there a multiple modes of action. For example, modeling to a low response level can be useful for estimating the response at doses where a high-dose mode of action would be less important.

If linear extrapolation is used, the assessment develops a candidate slope factor or unit risk for each suitable data set. These results are arrayed, using common dose metrics, to show the distribution of relative potency across various effects and experimental systems. The assessment then derives or selects an overall slope factor and an overall unit risk for the agent, considering the various dose-response analyses, the study preferences discussed in Section 6, and the possibility of basing a more robust result on multiple data sets.

## 7.5. Considering susceptible populations and lifestages

The assessment analyzes the available information on populations and lifestages that may be particularly susceptible to each effect. A tiered approach is used (U.S. EPA, 2005a, §3.5).

1) If an epidemiologic or experimental study reports quantitative results for a susceptible population or lifestage, these data are analyzed to derive separate toxicity values for susceptible individuals.

2) If data on risk-related parameters allow comparison of the general population and susceptible individuals, these data are used to adjust the general-population toxicity values for application to susceptible individuals.

3) In the absence of chemical-specific data, the EPA has developed *age-dependent adjustment factors* for early-life exposure to potential carcinogens that have a mutagenic mode of action. There is evidence of early-life susceptibility to various carcinogenic agents, but most epidemiologic studies and cancer bioassays do not include early-life exposure. To address the potential for early-life susceptibility, the EPA recommends (U.S. EPA, 2005b, §5):

   - 10-fold adjustment for exposures before age 2 years.
   - 3-fold adjustment for exposures between ages 2 and 16 years.

## 7.6. Reference values and uncertainty factors

An *oral reference dose* or an *inhalation reference concentration* is an estimate of an exposure (including in susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime (U.S. EPA, 2002, §4.2). Reference values are typically calculated for effects other than cancer and for suspected carcinogens if a well characterized mode of action indicates that a necessary key event does not occur below a specific dose. Reference values provide no information about risks at higher exposure levels.

The assessment characterizes effects that form the basis for reference values as adverse, considered to be adverse, or a precursor to an adverse effect. For developmental toxicity, reproductive toxicity, and neurotoxicity there is guidance on adverse effects and their biologic markers (U.S. EPA, 1998, 1996, 1991).

To account for uncertainty and variability in the derivation of a lifetime human exposure where adverse effects are not anticipated to occur, reference values are calculated by applying a series of *uncertainty factors* to the point of departure. If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level or a lowest-observed-adverse-effect level is used instead. The assessment discusses scientific considerations involving several areas of variability or uncertainty.

**Human variation.** The assessment accounts for variation in susceptibility across the human population and the possibility that the available data may not be representative of individuals who are most susceptible to the effect. A factor of 10 is generally used to account for this variation. This factor is reduced only if the point of departure is derived or adjusted specifically for susceptible individuals (not for a general population that includes both susceptible and non-susceptible individuals) (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991, §3.4).

**Animal-to-human extrapolation.** If animal results are used to make inferences about humans, the assessment adjusts for cross-species differences. These may arise from differences in toxicokinetics or toxicodynamics. Accordingly, if the point of departure is standardized to equivalent human terms or is based on toxicokinetic or dosimetry modeling, a factor of $10^{1/2}$ (rounded to 3) is applied to account for the remaining uncertainty involving toxicokinetic and toxicodynamic differences. If a biologically based model adjusts fully for toxicokinetic and toxicodynamic differences across species, this factor is not used. In most other cases, a factor of 10 is applied (U.S. EPA, 2011; 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991, §3.4).

**Adverse-effect level to no-observed-adverse-effect level.** If a point of departure is based on a lowest-observed-adverse-effect level, the assessment must infer a dose where such effects are not

expected. This can be a matter of great uncertainty, especially if there is no evidence available at lower doses. A factor of 10 is applied to account for the uncertainty in making this inference. A factor other than 10 may be used, depending on the magnitude and nature of the response and the shape of the dose-response curve (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991, §3.4).

**Subchronic-to-chronic exposure**. If a point of departure is based on subchronic studies, the assessment considers whether lifetime exposure could have effects at lower levels of exposure. A factor of 10 is applied to account for the uncertainty in using subchronic studies to make inferences about lifetime exposure. This factor may also be applied for developmental or reproductive effects if exposure covered less than the full critical period. A factor other than 10 may be used, depending on the duration of the studies and the nature of the response (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1994, §4.3.9.1).

**Incomplete database.** If an incomplete database raises concern that further studies might identify a more sensitive effect, organ system, or lifestage, the assessment may apply a database uncertainty factor (U.S. EPA, 2002, §4.4.5; 1998, §4.2; 1996, §4; 1994, §4.3.9.1; 1991, §3.4). The size of the factor depends on the nature of the database deficiency. For example, the EPA typically follows the suggestion that a factor of 10 be applied if both a prenatal toxicity study and a two-generation reproduction study are missing and a factor of $10^{1/2}$ if either is missing (U.S. EPA, 2002, §4.4.5).

In this way, the assessment derives candidate values for each suitable data set and effect that is credibly associated with the agent. These results are arrayed, using common dose metrics, to show where effects occur across a range of exposures (U.S. EPA, 1994, §4.3.9).

The assessment derives or selects an *organ- or system-specific reference value* for each organ or system affected by the agent. The assessment explains the rationale for each organ/system-specific reference value (based on, for example, the highest quality studies, the most sensitive outcome, or a clustering of values). By providing these organ/system-specific reference values, IRIS assessments facilitate subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site or through common mechanisms (NRC, 2009).

The assessment then selects an overall reference dose and an overall reference concentration for the agent to represent lifetime human exposure levels where effects are not anticipated to occur. This is generally the most sensitive organ/system-specific reference value, though consideration of study quality and confidence in each value may lead to a different selection.

## 7.7. Confidence and uncertainty in the reference values

The assessment selects a standard descriptor to characterize the level of confidence in each reference value, based on the likelihood that the value would change with further testing. Confidence in reference values is based on quality of the studies used and completeness of the database, with more weight given to the latter. The level of confidence is increased for reference values based on human data supported by animal data (U.S. EPA, 1994, §4.3.9.2).

**High confidence:** The reference value is not likely to change with further testing, except for mechanistic studies that might affect the interpretation of prior test results.

**Medium confidence:** This is a matter of judgment, between high and low confidence.

**Low confidence:** The reference value is especially vulnerable to change with further testing.

These criteria are consistent with guidelines for systematic reviews that evaluate the quality of evidence. These also focus on whether further research would be likely to change confidence in the estimate of effect (Guyatt et al., 2008b).

All assessments discuss the significant uncertainties encountered in the analysis. The EPA provides guidance on characterization of uncertainty (U.S. EPA, 2005a, §3.6). For example, the discussion distinguishes model uncertainty (lack of knowledge about the most appropriate experimental or analytic model) and parameter uncertainty (lack of knowledge about the parameters of a model). Assessments also discuss human variation (interpersonal differences in biologic susceptibility or in exposures that modify the effects of the agent).

August 2013

# EXECUTIVE SUMMARY

***Occurrence and Health Effects***

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a synthetic chemical used primarily as a military explosive. RDX releases have been reported in air, water, and soil. Exposure to RDX 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 ingestion of crops irrigated with contaminated water. Inhalation or dermal exposures are more likely in occupational settings.

Epidemiological studies provide only limited information on occupational populations exposed to RDX; several case reports describe effects primarily in the nervous system following acute exposure to RDX. Animal studies demonstrate toxicity, including nervous system effects, kidney and other urogenital effects, and male reproductive effects.

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.

**Effects Other Than Cancer Observed Following Oral Exposure**

EPA identified nervous system effects as a human hazard of RDX exposure. Several human case reports and animal studies provide consistent evidence of associations between RDX exposure and effects on the nervous system, including seizures or convulsions. Increased mortality was generally observed at RDX doses that induced nervous system effects, and several studies documented that deaths in most cases were preceded by tremors and convulsions. Although mechanistic data are insufficient to establish a mode of action (MOA) for RDX-induced convulsions, the available information suggests that nervous system effects are mediated by RDX binding to the picrotoxin convulsant site of the $GABA_A$ channel, resulting in disinhibition that leads to the onset of seizures.

EPA identified kidney and other urogenital effects as a potential human hazard of RDX exposure based on observations in 2-year studies of increased relative kidney weights in male and female mice and histopathological changes in the urogenital system of male rats exposed to RDX. An increased incidence of suppurative prostatitis was identified, and is considered a marker for RDX-related urogenital effects. There is no established MOA for RDX-related effects on the urogenital system.

Based on the finding of testicular degeneration in male mice exposed to RDX in diet for 2 years, in the only mouse study conducted of that duration, EPA identified suggestive evidence of

male reproductive effects as a potential human hazard of RDX exposure. There is no known MOA for male reproductive effects of RDX exposure.

Evidence for effects on other organs/systems, including the liver and developmental effects, was more limited than for the endpoints summarized above. EPA concluded that the evidence does not support effects on other organs/systems, including liver and developmental effects, as a potential human hazard of RDX exposure.

### 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 RfDs and proposed overall RfD for RDX**

| Effect | Basis | RfD (mg/kg-day) | Study exposure description | Confidence |
|---|---|---|---|---|
| Nervous system | Convulsions | $9 \times 10^{-4}$ | Subchronic | Medium |
| Kidney/urogenital | Suppurative prostatitis | $2 \times 10^{-3}$ | Chronic | Low |
| Male reproductive | Testicular degeneration | $2 \times 10^{-2}$ | Chronic | Low |
| **Proposed overall RfD** | **Nervous system effects** | **$9 \times 10^{-4}$** | **Subchronic** | **Medium** |

The overall RfD (see Table ES-2) is derived to be protective of all types of hazards associated with RDX exposure. 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 it represents the most sensitive human hazard of RDX exposure. Incidence of seizures or convulsions as observed in a subchronic gavage study (Crouse et al., 2006) was selected for derivation of the overall RfD as the study was well-conducted, utilized a more pure form of test material than other studies, and had five closely-spaced dose groups that allowed characterization of the dose-response curve. Benchmark dose (BMD) modeling was utilized to derive the point of departure (POD) for RfD derivation (expressed as the $BMDL_{01}$). A 1% response level was chosen because of the severity of the endpoint; this is supported by the observation in Crouse et al. (2006) that for all the dose groups where unscheduled deaths were recorded, mortality was strongly associated with convulsions. A physiologically-based pharmacokinetic (PBPK) model was used to extrapolate the $BMDL_{01}$ to a human equivalent dose (HED) based on RDX arterial blood concentration, which was then used for RfD derivation.

The proposed overall RfD was calculated by dividing the $BMDL_{01\text{-}HED}$ for nervous system effects by a composite uncertainty factor of 300 to account for extrapolation from animals to

humans (3), interindividual differences in human susceptibility (10), extrapolation of results from a subchronic study to a chronic study (3), and deficiencies in the toxicity database (3).

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

| Critical effect | Point of departure* | UF | Chronic RfD |
|---|---|---|---|
| Nervous system effects (convulsions) 90-d F344 rat study Crouse et al. (2006) | $BMDL_{01\text{-}HED}$: 1.3 mg/kg-d | 300 | $9 \times 10^{-4}$ mg/kg-d |

*A benchmark response (BMR) of 1% was used to derive the BMD and BMDL given the severity of the endpoint. The resulting POD was converted to a $BMDL_{01\text{-}HED}$ using a PBPK model based on modeled arterial blood concentration. The concentration was derived from the area under the curve (AUC) of modeled RDX concentration in arterial blood, which reflects the average blood RDX concentration for the exposure duration normalized to 24 hours.

### Effects Other Than Cancer Observed Following Inhalation Exposure

No studies were identified that provided useful information on 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.

### Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

An RfC for RDX could not be derived based on the available health effects data. Additionally, a PBPK model for inhaled RDX is not available to support route-to-route extrapolation from the RfD.

### Evidence for Human Carcinogenicity

Under EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), the database for RDX provides "suggestive evidence of carcinogenic potential" based on the finding of statistically significant trends for hepatocellular adenomas or carcinomas and alveolar/bronchiolar adenomas or carcinomas in female, but not male, $B6C3F_1$ mice (Lish et al., 1984). This is further supported by the finding of a statistically significant trend for hepatocellular carcinomas in male, but not female, F344 rats (Levine et al., 1983) exposed to RDX in the diet for two years. On the other hand, there was no evidence of carcinogenicity in Sprague-Dawley rats in a 2-year dietary study of RDX (Hart, 1976). No human studies are available to assess the carcinogenic potential of RDX. The MOA for liver and lung tumors in experimental animals is not known. Available in vitro and in vivo genotoxicity assays were largely negative for RDX, suggesting that parent RDX does not interact

directly with DNA. *N*-nitroso metabolites of RDX generated anaerobically have tested positive in some genotoxicity assays; their contribution to the overall carcinogenic potential of RDX is not known.

**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 two-year dietary study was generally well conducted, with four dose groups and adequate numbers of animals per dose group (85/sex/group, with interim sacrifices of 10/sex/group at 6 and 12 months), and included 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.

Although EPA concluded that there is "suggestive evidence of carcinogenic potential" for RDX, the Agency determined that quantitative analysis of the mouse tumor data may be useful for providing a sense of the magnitude of potential carcinogenic risk.

EPA calculated a single oral slope factor (OSF) that considered the combination of tumors. Point of departure (i.e., BMD and BMDL) estimates that corresponded to a specific risk of incidence of either of the tumors (liver or lung) were calculated. The single $BMDL_{10}$ so derived from the mouse tumors was extrapolated to the HED using $BW^{3/4}$ scaling, and an OSF was derived by linear extrapolation from the $BMDL_{10\text{-HED}}$. The OSF is $4 \times 10^{-2}$ per mg/kg-day, based on the liver and lung tumor response in female mice (Lish et al., 1984).

**Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure**

The carcinogenic potential of RDX by inhalation has not been investigated. A PBPK model to support route-to-route extrapolation of an inhalation unit risk based on oral carcinogenicity data was not available.

**Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes**

Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Lifestage, and in particular childhood susceptibility, has not been observed 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. Data to suggest males may be more susceptible than females to noncancer toxicity associated with RDX exposure are limited. Specifically, urogenital effects have been noted at lower doses than in females. Some evidence suggests 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 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.

**Key Issues Addressed in Assessment**

In most instances, the spectrum of effects associated with chemical exposure will range in severity, with relatively less severe effects generally occurring at doses lower than those associated with more severe or "frank" toxicity. Convulsions in rats were selected as the basis for derivation of the RDX RfD; less severe nervous system effects were generally not observed at lower doses. U.S. EPA (2012a) emphasizes that when modeling a dose-response relationship from a given set of data, statistical and biological characteristics of the dataset must be considered, including consideration of the severity of the effect. For convulsions, because of the severity of the effect itself and the strong association with mortality, a benchmark response (BMR) level of 1% was selected for modeling, balancing the quantitative limitations of the available animal bioassays and the severity of the effect. Use of a BMR of 1% extra risk of convulsions resulted in extrapolation below the range of experimental data and could potentially increase uncertainty in the BMD and BMDL values.

The candidate RfD for kidney and other urogenital effects is based on suppurative prostatitis. This organ/system-specific RfD is based on a dose-related increase in suppurative prostatitis as reported in a 2-year feeding study in male F344 rats (Levine et al., 1983), the only 2-year study in rats that examined the prostate. Some reports have hypothesized that the observed suppurative prostatitis was secondary to a bacterial infection unrelated to RDX toxicity (ATSDR, 2012; Sweeney et al., 2012a; Crouse et al., 2006). In reviewing the findings in Levine et al. (1983), EPA concluded that while an opportunistic bacterial infection may have been the proximal cause of the suppurative prostatitis, the infection was secondary to urogenital effects associated with RDX exposure. Histopathological findings for the bladder are not definitive because the design of the principal study called for histopathological examination of the bladder only if gross abnormalities were observed. Although the pathogenesis of kidney and urogenital effects is unclear, suppurative prostatitis was considered to be a marker for the broader array of kidney and other urogenital effects observed by Levine et al. (1983).

# LITERATURE SEARCH STRATEGY | STUDY SELECTION AND EVALUATION

**Literature Search and Screening Strategy**

A literature search and screening strategy was applied to identify literature related to characterizing the health effects of RDX. This strategy consisted of a search of online scientific databases and other sources, casting a wide net in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the health effects of RDX, and remaining references were sorted into categories for further evaluation.

The literature search for RDX was conducted through January 2014 using the databases and general keywords listed in Table LS-1 (see Appendix B for further details of the literature search strategy). More specifically, the literature search for RDX was conducted in four online scientific databases—Pubmed, Toxline, Toxcenter, and TSCATS. The detailed search approach for these databases, including the search strings and number of citations identified per database, is provided in Appendix B, Table B-1. Given the military applications of RDX, the Defense Technical Information Center (DTIC) database, a central online repository of defense-related scientific and technical information within the Department of Defense, was also searched. A separate strategy was applied in searching DTIC because of limitations in the classification and distribution of materials in DTIC; the detailed search strategy is described in Appendix B, Table B-2. This search of the five online databases identified 995 citations (after electronically eliminating duplicates). The computerized database searches were supplemented by review of online regulatory sources, "forward" and "backward" searches of Web of Science (Appendix B, Table B-3), as well as additional references added during development of the toxicological review (including guidance documents and other references that provide context for evaluating RDX health effects); 113 citations were obtained using these additional search strategies. In total, 1108 citations were identified using online scientific databases and additional search strategies.

EPA requested public submissions of additional information in 2010 (75 FR 76982; December 10, 2010); no submissions were received in response to this call for data. Additionally, EPA issued a request to the public for additional information in a Federal Register Notice in 2013 (78 FR 48674; August 9, 2013), and established a docket for public comment (EPA-HQ-ORD-2013-0430; available at www.regulations.gov) maintained through the development of the assessment.

**Table LS-1. Overview of the search strategy employed for RDX**

| Database | Keywords |
|---|---|
| Pubmed<br>Toxline<br>TSCATS1<br>Toxcenter<br>DTIC<br>WOS (forward and backward search only) | Chemical CASRN: 121-82-4<br>Synonyms: Cyclonite OR RDX OR Cyclotrimethylenetrinitramine OR "cyclotrimethylene trinitramine" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazine" OR "Hexahydro-1,3,5-trinitro-s-triazine" OR Hexogen OR "1,3,5-trinitro-1,3,5-triazine" OR "1,3,5-Triaza-1,3,5-trinitrocyclohexane" OR "1,3,5-Trinitro-1,3,5-triazacyclohexane" OR "1,3,5-Trinitrohexahydro-1,3,5-triazine" OR "1,3,5-Trinitrohexahydro-s-triazine" OR "1,3,5-Trinitroperhydro-1,3,5-triazine" OR "Esaidro-1,3,5-trinitro-1,3,5-triazina" OR "Hexahydro-1,3,5-trinitro-1,3,5-triazin" OR "Perhydro-1,3,5-trinitro-1,3,5-triazine" OR Cyclotrimethylenenitramine OR Trimethylenetrinitramine OR "Trimethylene trinitramine" OR Trimethyleentrinitramine OR "Trinitrocyclotrimethylene triamine" OR Trinitrotrimethylenetriamine OR "CX 84A" OR Cyklonit OR Geksogen OR Heksogen OR Hexogeen OR Hexolite OR "KHP 281" OR "PBX (af) 108" OR "PBXW 108(E)" OR "Pbx(AF) 108"<br>Synonym and CASRN search for all databases; Toxcenter, Pubmed, and WOS limited using toxicity-related keywords<br><br>Toxicity-related terms (see Appendix B for specific keywords)<br>Toxicity (including duration, effects to children and occupational exposure); development; reproduction; teratogenicity; exposure routes; pharmacokinetics; toxicokinetics; metabolism; body fluids; endocrinology; carcinogenicity; genotoxicity; antagonists; inhibitors |
| ChemID<br>TSCATS 2 & 8e submissions | Searched by CASRN |

The citations identified using the search strategy described above were screened using the title, abstract, and in limited instances, full text for pertinence to examining the health effects of RDX exposure. The process for screening the literature is described below and is shown graphically in Figure LS-1.[1]

- 21 references were identified as potential sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays.

- 65 references were identified as supporting studies; these included 16 studies describing physiologically-based pharmacokinetic (PBPK) models and other toxicokinetic information, 25 studies providing genotoxicity and other mechanistic information, 7 acute toxicity studies, and 17 human case reports. Studies investigating the effects of acute exposures and

[1] Studies were assigned (or "tagged") to a given category in HERO that best reflected the primary content of the study. Studies were not assigned multiple tags in order to simplify the tracking of references. Nevertheless, the inclusion of a citation in a given category (or tag) did not preclude its use in one or more other categories. For example, Woody et al. (1986), a case report of accidental ingestion of RDX by a child, was tagged to the human case reports under Supporting Studies in Figure LS-1. This case report also provides pharmacokinetic data and was a pertinent source of information on RDX toxicokinetics, but was not assigned a second tag for toxicokinetics.

case reports are generally less pertinent for characterizing health hazards associated with chronic oral and inhalation exposure. Therefore, information from these studies was not considered for extraction into evidence tables. Nevertheless, these studies were still evaluated as possible sources of supporting health effects information.

- 277 references were identified as secondary sources of health effects information (e.g., reviews and other agency assessments) or as studies providing potentially useful contextual information (e.g., studies providing information on exposure levels); these references were kept as additional resources for development of the Toxicological Review.

- 745 references were identified as not being pertinent to an evaluation of the health effects of RDX and were excluded from further consideration (see Figure LS-1 for exclusion categories).

The documentation and results for the literature search and screen can be found on the Health and Environmental Research Online (HERO) website (http://hero.epa.gov/index.cfm/project/page/project_id/2216).

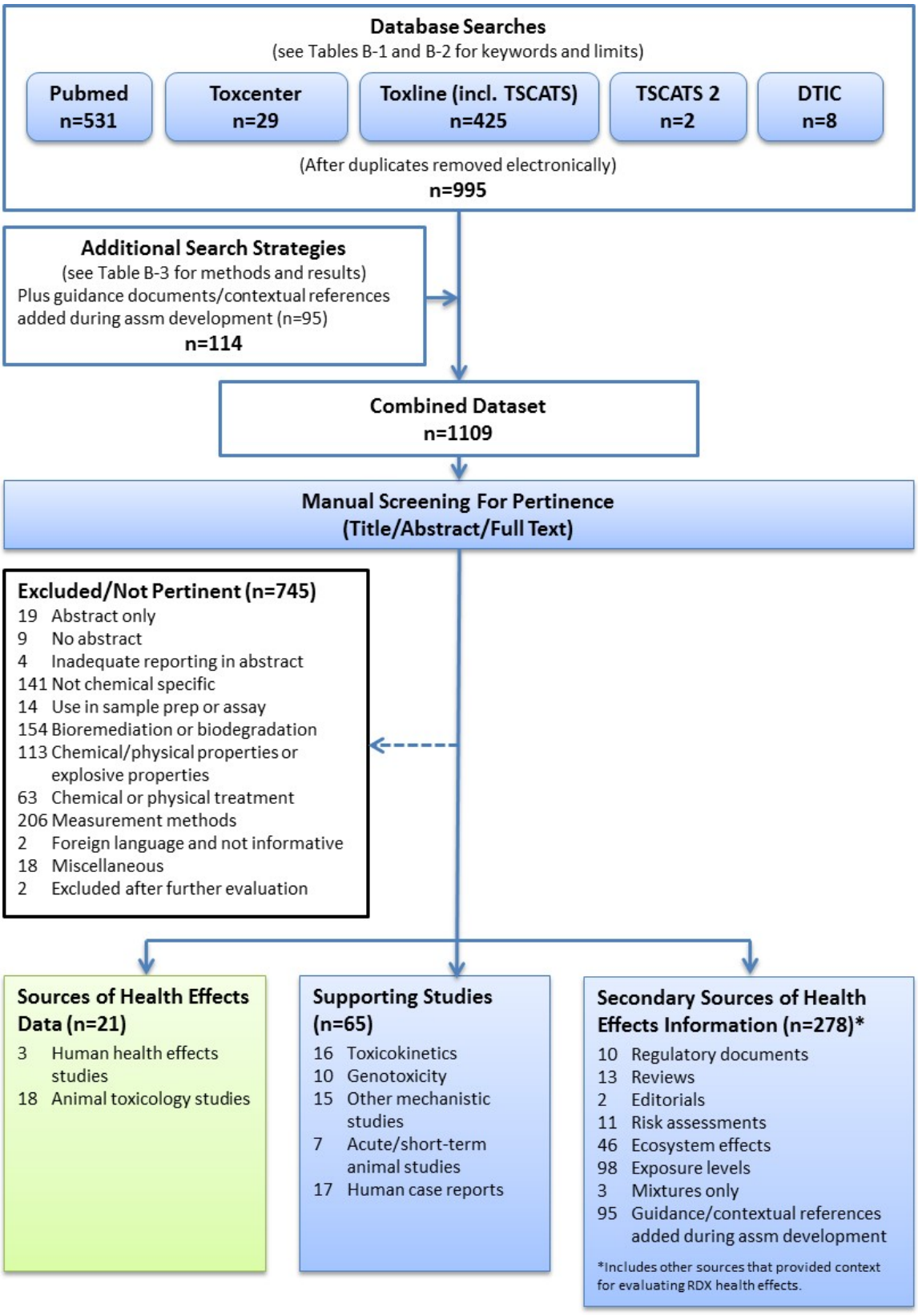



**Figure LS-1. Summary of literature search and screening process for RDX.**

## Selection of Critical Studies for Inclusion in Evidence Tables

### *Selection of Critical Studies*

The 21 studies retained after the literature search and screen (Figure LS-1) were evaluated for aspects of its design or conduct that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of hazard potential. Much of the key information for conducting this evaluation can generally be found in the study's methods section and in how the study results are reported. Importantly, the evaluation at this stage does not consider study results, or more specifically, the direction or magnitude of any reported effects.

To facilitate this evaluation, evidence tables were constructed that systematically summarize the important information from each study in a standardized tabular format as recommended by the NRC (2011). The studies selected for inclusion in evidence tables are critical for assessing the health effects of RDX. The evidence tables include all studies that inform the overall synthesis of evidence for hazard potential; in general, the goal in developing evidence tables is to be inclusive.

Studies were excluded from evidence tables if flaws in its design, conduct, or reporting are so great that the results would not be considered credible (e.g., studies where concurrent or essential historical control information is lacking). Such study design flaws are discussed in a number of EPA's guidelines (see http://www.epa.gov/iris/backgrd.html) or summarized in the Preamble. For RDX, four studies were considered uninformative and removed from further consideration in the assessment because of fundamental issues with study design, conduct, or reporting. The specific studies and basis for exclusion are summarized in Table LS-2.

**Table LS-2. Studies determined not to be informative because of significant issues with design, conduct, or reporting**

| Reference | Rationale for exclusion |
|---|---|
| Haskell Laboratories (1942); 14-wk study in dogs | Incomplete information on exposure levels; low numbers of animals; breed of dog not reported; inadequate reporting of results; sections of document illegible. |
| Von Oettingen et al. (1949); 10-wk oral study in rats | No control group; strain of rat was not reported. |
| ATSDR (1996); Disease prevalence study in residential population | Study of a population residing in two neighborhoods where RDX had been detected in well water. The study was conducted 7 yrs after residents were provided the opportunity to connect to a municipal water supply. Only one target-area household reported using private well water for bathing and cooking at the time of the health study. The study was not considered informative because the design was not able to adequately define the exposed population. |

| Reference | Rationale for exclusion |
|---|---|
| Unpublished report from the DTIC database;<br>Human and animal data | One section of the report describes a human case series with no referent group. Issues with the inhalation experimental animal studies included lack of control groups, low numbers of animals tested, incomplete information on exposure levels, and inadequate reporting of results. |

The health effects literature for RDX is not extensive. All human and experimental animal studies of RDX involving repeated exposure that were not identified as uninformative because of fundamental issues with study design, conduct, or reporting were considered in assessing the evidence for health effects associated with chronic exposure to RDX. These studies are considered the "critical" studies for which study methods and results are presented in evidence tables and exposure-response arrays.

Other health effect studies of RDX, including human case reports and experimental animal studies involving exposures of short-term duration or routes of exposure other than oral and inhalation, were not included in evidence tables. Nevertheless, these studies were considered, where relevant, in the evaluation of RDX health hazards.

**Study Evaluation**

In evaluating the evidence to determine whether RDX exposure may pose a hazard for each of the health effects considered in this assessment, methodological aspects of a study's design, conduct, and reporting were considered in the overall evaluation and synthesis of the pertinent data. In general the relevance and informativeness of the available studies were evaluated as outlined in the Preamble and in EPA guidance (e.g., *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* (U.S. EPA, 1994)). In addition, in 2012, EPA obtained external peer reviews of two 2-year bioassays (Lish et al., 1984; Levine et al., 1983) and one 90-day study (Crouse et al., 2006) that were available only as laboratory reports. The report of the peer reviews is available at www.epa.gov/hero (search for HERO ID 2519581).

The general findings of this evaluation are presented in the remainder of this section. Study evaluation considerations that are outcome specific are discussed in the relevant health effect sections in Section 1.1.

***Human Studies***

The body of literature on RDX includes three studies of populations occupationally exposed to RDX (one case-control and two cross-sectional studies) (West and Stafford, 1997; Ma and Li, 1992; Hathaway and Buck, 1977).

To varying degrees, these epidemiology studies of RDX are limited by their study design, uncertainty in estimates of exposure, inadequate reporting, and/or failure to account for potential confounding exposures. All three studies were based on a relatively small number of participants

(60–69 exposed workers in the cross-sectional studies and 32 cases in the case-control study). The study by Ma and Li (1992) of Chinese industrial workers provided limited information on participant recruitment, selection, and participation rate; information was not adequate to evaluate the potential for selection bias.

Of the three epidemiological studies, more detailed exposure information was collected by Hathaway and Buck (1977). Atmospheric and paired breathing zone sampling was performed; however, the paper included limited reporting of RDX concentrations in workplace air. Ma and Li (1992) reported mean RDX exposure concentrations (with standard deviations) for two exposure groups, but provided no information on the source of these concentrations or how monitoring was performed. In the case-control study by West and Stafford (1997), semi-quantitative exposure estimates (low, moderate, or high) were based on interviews with employees.

Ma and Li (1992) did not adjust for any potential risk factors, e.g., alcohol consumption. In the study by Hathaway and Buck (1977) that included evaluations of liver, renal, and hematology endpoints, workers with trinitrotoluene (TNT) exposure were appropriately excluded from the exposed groups, since TNT is another explosive that is associated with liver and hematological system toxicity. The case-control study by West and Stafford (1997), which examined hematology outcomes, did not perform statistical analyses to adjust for other risk factors or occupational exposures (including TNT). Further, the impact of age or gender could not be assessed as the cases and controls were not matched.

The methodological limitations in these three studies were considered in the synthesis of evidence for each of the health effects and in reaching determinations of hazard (see Section 1.1).

In addition to these three occupational epidemiology studies, the human health effects literature includes 16 case reports that describe effects following acute exposure to RDX. Case reports are often anecdotal and typically describe unusual or extreme exposure situations, providing little information that would be useful for characterizing chronic health effects. Therefore, RDX case reports were only briefly reviewed; a critical evaluation of these studies was not undertaken. A summary of these case reports is provided in Appendix C, Section C.3.

### *Experimental Animal Studies*

The oral toxicity database for RDX includes three chronic studies in rats and mice, eight subchronic studies in rats, mice, dogs, and monkeys, two short-term studies, and four reproductive/developmental toxicity studies in rats and rabbits (including a two-generation reproductive study). Only one inhalation study of RDX was identified. As discussed in Appendix B and Table LS-2, this inhalation study was considered uninformative and excluded from consideration in the development of the Toxicological Review because of study design issues (including lack of a control group, incomplete information on exposure levels, and inadequate reporting). Therefore, evaluation of the experimental animal database for RDX is limited to studies of oral toxicity. An evaluation of the oral toxicity literature, organized by general methodological features, is provided in the remainder of this section.

***Test animal***

The RDX database consists of health effect studies conducted in multiple strains of rats (F344, Sprague-Dawley, CD), mice ($B6C3F_1$), dog (beagle), and monkey. The species and strains of animals used are consistent with those typically used in laboratory studies. All of these species or strains were considered relevant to assessing the potential human health effects of RDX. Several studies in the RDX database provided inadequate information on test animals. The strain of monkey (rhesus or cynomolgus) used in the study by Martin and Hart (1974) was not clearly specified. In one study, the breed of dog and strain of rat were unreported (Von Oettingen et al., 1949). The species, strain, and sex of the animals used is recorded in the evidence tables.

Other studies of RDX were identified that used nonstandard species, including deer mice (*Peromyscus maniculatus*), western fence lizards (*Sceloporus occidentalis*), prairie voles (*Microtus ochrogaster*), and northern bobwhite quail (*Colinus virginianus*). These studies provide information relevant to RDX toxicokinetics and mechanism of action on the nervous system, but not health effects data. Therefore, these studies are not included in evidence tables, but are discussed where relevant in the assessment.

***Experimental setup***

General aspects of study design and experimental setup were evaluated for all studies that included health effect data to determine if they were appropriate for evaluation of specific endpoints. Key features of the experimental setup, including the periodicity and duration of exposure, timing of exposure (e.g., gestational days for developmental studies), experimental group sample sizes, and interim sacrifices are summarized in the evidence tables. Note that sample size was not a basis for excluding a study from consideration. For example, the informativeness of Hart (1974) and Martin and Hart (1974) was reduced in light of the small sample sizes in each study (3/sex/group), but the studies would still inform the consistency of effects observed for a specific endpoint across species (dog and monkey). Elements of the experimental setup that could influence interpretation of study findings are discussed in the relevant hazard identification sections of the assessment.

***Exposure***

Properties of the test material were also considered in determining whether the exposures were sufficiently specific to the compound of interest. Two properties of the RDX test materials that varied across experimental animal studies and that were taken into consideration in evaluating RDX hazard are the particle size and purity of the test material. The purity of RDX used in health effects studies varied from 84-99.99%. The major contaminants were octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) and water, which are the primary contaminants of RDX produced through the Bachmann process. The majority of studies used RDX with ~10% impurities; only Crouse et al. (2006) used 99.99% pure RDX as a test material in their study. The toxicity of HMX was reviewed by the IRIS Program in 1988 (www.epa.gov/iris); histopathological changes in the

liver in male F344 rats and in the kidney in female rats were reported in a 13-week feeding study. No chronic studies were available to evaluate the carcinogenicity of HMX. The presence of the impurities introduces some uncertainty in attribution of toxicity to RDX. However, consistency in the doses at which some toxic effects were seen across studies suggests that the uncertainty associated with the use of less pure test materials may be relatively small. Evidence of neurotoxic effects in the study with 99.99% pure RDX occurred at doses of 8–15 mg/kg-day; studies with less pure RDX reported similar symptoms at doses of ≥20 mg/kg-day. It should be noted that the test materials employed in these studies are considered representative of RDX that would be released into the environment.

Differences in milling procedures used to generate the test material resulted in the use of RDX of varying particle sizes across studies. Some studies utilized a relatively fine particle size (majority of particles were <66 μm in size) while others used test material with comparatively coarse particle size (~200 μm particle size). Differences in particle size across studies could result in different rates of absorption of RDX into the blood stream, which could account for differences in some of the toxicities observed across studies, including neurotoxicity. Information on test material purity and particle size as provided by study authors is reported in the evidence tables, and was considered in evaluating the toxicity of RDX. Lack of characterization of the test material in the studies by Hart (1974), Hart (1976), and Martin and Hart (1974) was considered a deficiency.

***Endpoint evaluation procedures***

Some methodological considerations used to evaluate studies of RDX toxicity are outcome specific—in particular effects on the nervous system and development. Outcome-specific methodological considerations are discussed in the relevant health effect sections in Section 1.1. For example, many of the studies that noted neurotoxicity in the form of seizures or convulsions were not designed to assess that specific endpoint and reported number animals with seizures anecdotally. While these studies can provide qualitative evidence of neurotoxicity, they may have underestimated the true incidence of seizures or convulsions because they were not designed to systematically evaluate neurotoxic outcomes.

***Outcomes and data reporting***

In evaluating studies, consideration was given to whether data were reported for all pre-specified endpoints and study groups, and whether any data were excluded from presentation or analysis. For example, it was noted where histopathological analysis was limited to control and high-dose groups, a study reporting feature that limited the ability to identify dose-related trends. In limited cases, EPA performed additional statistical analysis to identify trends or refine analyses consistent with EPA guidance (e.g., analyzing developmental data sets on a per litter basis rather than individual fetus). Data from studies have been extracted and presented in evidence tables.

***Notable features of the RDX database***

Three two-year toxicity bioassays of RDX are available as unpublished laboratory studies. The bioassays by Levine et al. (1983) in the rat and by Lish et al. (1984) in the mouse were conducted in accordance with Food and Drug Administration (FDA) Good Laboratory Practices (GLPs) in place at the time of the studies. Both studies included interim sacrifices (at 6 and 12 months). Complete histopathological examinations were performed on all animals in the control and high-dose groups; however, only a subset of tissues was examined in the mid-dose groups, limiting the ability to identify dose-related trends for tissues with incomplete histopathology. In the mouse bioassay by Lish et al. (1984), the initial high dose (175 mg/kg-day) was reduced to 100 mg/kg-day at week 11 because of high mortality, thereby reducing the number of high-dose animals on study for the full 2 years of dosing (see Table LS-3). Because they were available only as laboratory reports, peer reviews of the Levine et al. (1983) and Lish et al. (1984) studies were conducted by EPA in 2012. The peer reviewers generally concluded that the reports provided useful information on the toxicity of RDX, noting that there were limitations in interpretation due to the histopathological analysis and the statistical approaches employed in the reports. An earlier two-year study in the rat by Hart (1976) used a dose range that was lower than the subsequent studies (high dose of 10 mg/kg-day), and that may not have been sufficient to elicit some effects in treated animals. Histopathology findings were limited by the lack of pathology examinations in the mid-dose groups and the lack of individual time of death, which impacts the ability to interpret the histopathology data. In addition, a heating system malfunction on days 75 - 76 of the study resulted in the death of 59 rats from the control and treatment groups, thereby reducing the number of animals on study (see Table LS-3).

Short-term and subchronic toxicity studies of RDX were published or reported between the years 1949 and 2006, and differences in robustness of study design, conduct, and reporting reflects that range. All but one of the eight short-term and subchronic toxicity studies of RDX are available as unpublished laboratory studies; only Von Oettingen et al. (1949) was published. The majority of studies conducted histopathological examinations on only some of the experimental groups (e.g., control and high dose). One subchronic study Crouse et al. (2006) was peer-reviewed by EPA in 2012. The peer reviewers determined that the report provided useful information on the toxicity of RDX, including an array of endpoints for neurotoxicity and immunotoxicity. Limitations in the study were based on an incomplete understanding of the neurotoxicity that may have been resolved with more histological evaluation as well additional behavioral assessment.

Some of the more important limitations in study design, conduct, and reporting of experimental animal toxicity studies of RDX are summarized in Table LS-3. Limitations of these studies were taken into consideration in evaluating and synthesizing the evidence for each of the health effects in Section 1.1.

**Table LS-3. Experimental animal studies considered less informative because of certain study design, conduct, or reporting limitations**

| References | Study design, conduct, and reporting limitations |
|---|---|
| Lish et al. (1984); Levine et al. (1984)<br>2-yr mouse study | The initial high dose (175 mg/kg-d) was reduced to 100 mg/kg-d at wk 11 due to high mortality. Mortality of surviving mice was similar to controls after dose reduction. |
| Hart (1976)<br>2-yr rat study | A heating system malfunction on d 75−76 of the study resulted in the deaths of 59 rats from the control and treatment groups. Dead animals were subsequently eliminated from the analysis. There were still more than 80 rats/sex/group after the overheating incident, and ≥50 rats/sex/group at termination.<br>Histopathology findings were limited by the lack of pathology examinations in the mid-dose groups and the lack of individual time of death, which impacts the ability to interpret the histopathology data. |
| Cholakis et al. (1980)<br>13-wk mouse study (Experiment 1) | Dose range was too low to produce effects in mice. Histopathological examinations were not performed. |
| Cholakis et al. (1980)<br>13-wk mouse study (Experiment 2) | Nonstandard dosing regimen followed: 0, 40, 60, 80 mg/kg-d for 2 wks. For the next 11 wks, the dosing was inverted, so that the 40 mg/kg-d group received 320 mg/kg-d, the 60 mg/kg-d group received 160 mg/kg-d, and the 80 mg/kg-d group continued to receive the same dose. The rationale for this dosing regimen was not provided in the study report. |
| Von Oettingen et al. (1949)<br>12-wk rat study | The strain of rat was not reported. Only gross observations made at autopsy. |
| Von Oettingen et al. (1949)<br>6-wk dog study | The breed of dog was not reported. Only gross observations made at autopsy. |
| Martin and Hart (1974)<br>90-d monkey study | Species of monkey is unclear (either Cynomolgus or Rhesus). Some test subjects may have had variable dosing due to emesis. Small sample size per dose group (n=3). |

# 1. HAZARD IDENTIFICATION

## 1.1. PRESENTATION AND SYNTHESIS OF EVIDENCE BY ORGAN/SYSTEM

### 1.1.1. Nervous System Effects

Nervous system effects following RDX exposure have been observed in multiple case reports, and the association between RDX exposure and neurobehavioral effects has been examined in a single occupational epidemiology study. Information relevant to an examination of the association between RDX exposure and nervous system effects also comes from experimental animal studies involving chronic, subchronic, and gestational exposure to ingested RDX. A summary of nervous system effects associated with RDX exposure is presented in Tables 1-1 and 1-2 and Figure 1-1.

In a cross-sectional study by Ma and Li (1992), neurobehavioral effects were evaluated in Chinese workers occupationally exposed to RDX. Memory retention and block design scores[2] were significantly lower among exposed workers (mean concentrations of RDX in two exposed groups: 0.407 and 0.672 mg/m$^3$) compared to unexposed workers from the same plant. However, no significant differences were observed between the groups on other neurobehavioral tests (e.g., simple and choice reaction times, block design, and letter cancellation test) (Table 1-1). This study did not consider potential confounders such as alcohol consumption or co-exposure to TNT.

Case reports support an association between RDX exposure and neurological effects (see Appendix C, Section C.3). Severe neurological disturbances include tonic-clonic seizures (formerly known as grand mal seizures) in factory workers (Testud et al., 1996b; Testud et al., 1996a; Kaplan et al., 1965; Barsotti and Crotti, 1949), seizures and convulsions in exposed soldiers serving in Vietnam (Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968), seizures, dizziness, headache and nausea following non-wartime/non-occupational exposures (Kasuske et al., 2009; Davies et al., 2007; Küçükardali et al., 2003; Hett and Fichtner, 2002; Harrell-Bruder and Hutchins, 1995; Goldberg et al., 1992), and seizures in a child following ingestion of plasticized RDX from the mother's clothing (Woody et al., 1986).

Nervous system effects in experimental animals, including seizures and convulsions (used interchangeably by study authors), tremors, behavioral changes, irritability, and hyperactivity, have been observed in the majority of chronic, subchronic, and developmental studies following oral exposure to RDX (see Table 1-2 and Figure 1-1). In a 2-year dietary study in F344 rats,

[2]The memory quotient index measured short-term hearing memory, visual memory, combined hearing and visual memory, and learning ability. The block design index measured visual perception and design replication, and the ability to analyze spatial relationships.

administration of 40 mg/kg-day RDX resulted in convulsions (Levine et al., 1983); convulsions were not observed at lower doses in the same study (≤8 mg/kg-day) (Levine et al., 1983) or in Sprague-Dawley rats following chronic dietary administration of 10 mg/kg-day, the highest dose tested (Hart, 1976). Convulsions were observed in B6C3$F_1$ mice exposed to RDX for 2 years at doses similar to or higher than those inducing convulsions in the rat (Levine et al., 1984; Lish et al., 1984). Subchronic dietary exposure was also associated with convulsions in the rat, although doses reported to increase convulsive activity were inconsistent across studies. Convulsions were reported in RDX-exposed rats at subchronic doses as low as 8 and 25 mg/kg-day (Crouse et al., 2006; Von Oettingen et al., 1949). In contrast, Levine et al. (1990) reported convulsions in rats following subchronic exposure only at a dose of 600 mg/kg-day; however, the unpublished technical report of this study (Levine et al., 1981a) inconsistently reported convulsions at 600 mg/kg-day and ≥30 mg/kg-day, thereby reducing confidence in the identification of the dose level at which nervous system effects are observed in this study. No evidence of seizures, convulsions or tremors was reported in three subchronic rat studies that used relatively lower doses of RDX (highest administered doses: 10–50 mg/kg-day) (MacPhail et al., 1985; Cholakis et al., 1980). RDX exposure (by gavage) during gestation in the rat was associated with induction of seizures or convulsions in the dams at doses ranging from 2 to 40 mg/kg-day (Angerhofer et al., 1986; Cholakis et al., 1980)—demonstrating that effects on the nervous system can be observed following exposure durations as short as 10–14 days. Convulsions were also reported in dogs exposed to 50 mg/kg-day RDX (Von Oettingen et al., 1949), but not 10 mg/kg-day (Hart, 1974), and in two of three monkeys of both sexes following a gavage dose of 10 mg/kg-day (Martin and Hart, 1974).

In the only study addressing susceptibility to seizures, Burdette et al. (1988) found that seizure occurrence was greater in Long Evans rats exposed to a single dose of 50 or 60 mg/kg RDX by gavage when challenged with an audiogenic stimulus 8 and 16 hours after treatment. However, no audiogenic seizures were observed at the earlier 2- and 4-hour post-dosing test periods even though RDX plasma concentrations were elevated throughout the testing period. In a complementary experiment, Long Evans rats treated daily with 6 mg/kg-day RDX for up to 18 days required fewer stimulation trials to exhibit amygdaloid kindled seizures compared to controls. Neither the purity nor the specific particle size of the RDX used in these experiments were reported.

The majority of animal studies reported convulsions and/or seizures as clinical observations; interpretation of these observations is limited to some extent because the nature and severity of convulsions and seizures were not more fully characterized. The 90-day study by Crouse et al. (2006) was one of the few studies that collected and reported incidence data for convulsions and tremors, and demonstrated a clear dose-related increase in convulsions and tremors in male and female F344 rats associated with RDX exposure via gavage (see Table 1-2). Tremors were reported following administration of ≥12 mg/kg-day, persisting throughout the 90-day study. Convulsions were observed at ≥8 mg/kg-day in male and female rats; information on duration and onset was not reported (Crouse et al., 2006). In general, gavage dosing (Crouse et al.,

2006; Cholakis et al., 1980) induced convulsions at lower doses than did dietary administration, possibly due to the bolus dosing resulting from gavage administration and the comparatively faster peak absorption of RDX.

Several experimental animal studies documented that unscheduled deaths were frequently preceded by convulsions or seizures. Crouse et al. (2006) stated that nearly all observed pre-term deaths in rats exposed to RDX for 90 days were preceded by neurotoxic signs such as tremors and convulsions. In a 2-year study in rats, Levine et al. (1983) observed that tremors and/or convulsions were often seen in high-dose animals prior to their death. Further, in a rat developmental study (Cholakis et al., 1980), investigators concluded that early deaths in dams were preceded by convulsions based on the observation of convulsions in one rat prior to death, and a similar appearance (e.g., dried blood round the mouth and nose) in other dams that died during the study. A few studies reported mortality that was not specifically or directly associated with neurological effects (Angerhofer et al., 1986; Levine et al., 1981a; Von Oettingen et al., 1949); however, in these studies, animals may not have been monitored for clinical observations with sufficient frequency to have observed convulsive activity prior to death.

Additional neurobehavioral effects associated with RDX exposure in rats included increased hyperactivity, hyper-reactivity, fighting, and irritability at doses similar to those that induced tremors, convulsions, and seizures (10–100 mg/kg-day) (Levine et al., 1990; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Von Oettingen et al., 1949). Hyperactivity and nervousness were also reported in male mice that received a subchronic exposure to 320 mg/kg-day RDX (Cholakis et al., 1980). No changes in motor activity, flavor aversion, scheduled-controlled behavior, or acoustic startle response were observed in a 30-day gavage study in rats, but doses were relatively low (≤10 mg/kg-day) (MacPhail et al., 1985), and no significant changes in behavioral or neuromuscular activity were observed in rats following exposure to ≤15 mg/kg-day for 90 days (Crouse et al., 2006). Crouse et al. (2006) concluded that stained haircoats and increased barbering in female F344 rats receiving 15 mg/kg-day may have been caused by the oral dosing procedure (gavage) alone.

Observations of changes in absolute and relative brain weight were mixed across studies. Among chronic oral studies, a decrease in absolute brain weight of female $B6C3F_1$ mice (3–4% relative to control) was reported at doses ≥35 mg/kg-day (Levine et al., 1984; Lish et al., 1984). Conversely, an increase in absolute brain weight of 2% relative to control was observed in F344 rats at 40 mg/kg-day in another two-year oral bioassay (Levine et al., 1983; Thompson, 1983). Similarly elevated absolute brain weights were reported in subchronic assays in $B6C3F_1$ mice and F344 rats (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980); however, the changes were not consistently observed across studies. Relative brain weights in some studies showed correspondingly greater increases compared to absolute brain weight (Crouse et al., 2006; Levine et al., 1983; Thompson, 1983; Cholakis et al., 1980), but these changes were likely a result of changes in body weight in the study, and were not a useful measure

of effects of RDX on brain weights. Based on an evaluation of the relationship between organ weight and body/brain weight to determine which endpoint (organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio) is likely to more accurately detect target organ toxicity, Bailey et al. (2004) concluded that brain weights are not modeled well by any of the choices, and that alternative analysis methods should be utilized.

Across the studies summarized in Table 1-2, nervous system responses to RDX did not show a predicted relationship with duration of exposure. For example, seizures or convulsions were observed in F344 rats in some subchronic studies at doses lower than in studies of chronic duration, and at even lower doses in dams exposed for approximately 2 weeks during gestation. In some studies, seizures appeared soon after dosing, suggesting that seizure induction was more strongly correlated with dose level rather than with duration of exposure. Williams et al. (2011) demonstrated that RDX is rapidly absorbed and crosses the blood-brain barrier following oral administration in rats, and that distribution of low levels of RDX (8 µg/g ww) to the brain correlated with seizure onset.

Similarly, nervous system effects across studies did not show a consistent relationship with dose. This lack of consistency may, at least in part, be attributed to differences in the purity or particle size of the test material across studies. Assuming that increased particle size results in slowed absorption and distribution to the brain, studies that used a larger particle size may be expected to produce less neurotoxicity in test animals. The mouse study by Cholakis et al. (1980) used a relatively large RDX particle size (200 µm) compared to the rat study by Levine et al. (1983) that used a smaller (<66 µm) particle size. This could contribute to why the Cholakis et al. (1980) subchronic dietary study in the mouse (doses up to 320 mg/kg-day RDX) and rat (doses up to 40 mg/kg-day) failed to report seizures or convulsions. Finally, differences in study design may have contributed to differences in reported neurological responses in subchronic and chronic duration studies; in particular, the number of daily observations for clinical signs may not have been sufficiently frequent to provide an accurate measure of the incidence of seizures or other nervous system effects.

**Table 1-1. Evidence pertaining to nervous system effects in humans**

| Reference and study design | Results | | | |
|---|---|---|---|---|
| Ma and Li (1992) (China)<br>Cross-sectional study, 60 workers exposed to RDX (30 in Group A [26 males; 4 females]; 30 in Group B [24 males; 6 females]), compared to 32 workers with similar age, education level, and length of employment from same plant with no exposure to RDX (27 males; 5 females).<br>**Exposure measures:** Details of exposure measurement were not provided; exposed workers were divided into two groups based on RDX concentration in the air:<br>**Concentration** (mg/m$^3$)<br>Group A 0.407 (± 0.332)<br>Group B 0.672 (± 0.556)<br>**Effect measures**[a]: Five neurobehavioral function tests and five additional memory subtests.<br>**Analysis**: Variance (F-test); unadjusted linear regression, multiple regression, and correlation analysis. | **Neurobehavioral function tests, scaled scores** *(mean, standard deviation)* | | | |
| | Test | Control | Group A | Group B |
| | Memory retention* | 111.3 (9.3) | 96.9 (9.6) | 91.1 (10.3) |
| | Simple reaction time (milliseconds) | 493 (199) | 539 (183) | 578 (280) |
| | Choice reaction time (milliseconds) | 763 (180) | 775 (161) | 770 (193) |
| | Block design* (elapsed time) | 18.0 (5.4) | 16.0 (4.3) | 13.5(6.7) |
| | Letter cancellation (quality per unit time) | 1,487 (343) | 1,449 (331) | 1,484 (443) |
| | *$p < 0.01$ (overall F-test); no statistically significant differences between Group A and Group B.<br>Lower score indicates worse performance. | | | |
| | **Memory retention subtests, scaled scores** *(mean, standard deviation)* | | | |
| | Subtest | Control | Group A | Group B |
| | Directional memory* | 23.5 (3.6) | 17.2 (4.9) | 18.1 (5.7) |
| | Associative learning* | 24.9 (5.1) | 20.0 (4.3) | 18.5 (4.6) |
| | Image free recall* | 24.1 (3.8) | 20.9 (4.1) | 20.4 (3.3) |
| | Recognition of nonsense pictures* | 26.3 (3.6) | 23.2 (4.9) | 21.6 (4.3) |
| | Associative recall of portrait characteristics* | 26.3 (3.3) | 20.3 (4.4) | 18.5 (4.3) |
| | *$p < 0.01$ (overall F-test); no statistically significant differences between Group A and Group B.<br>Lower score indicates worse performance.<br>Total behavioral score negatively correlated with exposure index (high exposure correlated with poor performance). | | | |

[a]Symptom data were not included in evidence table because of incomplete reporting.

**Table 1-2. Evidence pertaining to nervous system effects in animals**

| Reference and study design | Results |
|---|---|
| *Convulsions and neurobehavioral effects* | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | One male mouse in the 35 mg/kg-d dose group and one female mouse in the 175/100 mg/kg-d group convulsed near the end of the study. |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | No neurological effects, as evidenced by clinical signs or changes in appearance or behavior, were reported. |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Tremors, convulsions, and hyper-responsiveness to stimuli were noted at 40 mg/kg-d; no incidence data were reported. |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants<br>~200 µm particle size<br>0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[b]<br>Diet<br>13 wks | Hyperactivity and/or nervousness observed in 50% of the high-dose males; no signs observed in females[a]; no incidence data were reported. |

| Reference and study design | Results |
|---|---|
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | No neurological effects, as evidenced by clinical signs or changes in appearance or behavior, were reported. |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study;<br>F0: 22/sex/group; F1: 26/sex/group;<br>F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | No neurological effects were reported. |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses: 0 / 4 / 8[a] / 10 / 12 / 15<br>**Convulsions** *(incidence)*<br>M: 0/10 / 0/10 / 1/10 / 3/10 / 8/10 / 7/10<br>F: 0/10 / 0/10 / 2/10 / 3/10 / 5/10 / 5/10<br>**Tremors** *(incidence)*<br>M: 0/10 / 0/10 / 0/10 / 0/10 / 2/10 / 3/10<br>F: 0/10 / 0/10 / 0/10 / 0/10 / 0/10 / 1/10 |
| Levine et al. (1981a); Levine et al. (1990); Levine et al. (1981b)[d]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Hyper-reactivity to approach was observed in groups receiving ≥100 mg/kg-d; no incidence data were reported.<br>Tremors and convulsions were observed prior to death in some animals receiving 600 mg/kg-d; no incidence data were reported.[c] |
| Von Oettingen et al. (1949)<br>Rats, sex/strain not specified, 20/group<br>90–97% pure, with 3–10% HMX; particle size not specified<br>0, 15, 25, or 50 mg/kg-d<br>Diet<br>3 mo | Hyperirritability and convulsions were observed in the 25 and 50 mg/kg-d groups[a]; no incidence data were reported. |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | No neurological effects, as evidenced by clinical signs or changes in appearance or behavior, were reported. | | | | |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 0.1 | 1 | 10[a] |
| | **CNS effects characterized as trembling, shaking, jerking, or convulsions** *(incidence)* | | | | |
| | M | 0/3 | 0/3 | 0/3 | 2/3 |
| | F | 0/3 | 0/3 | 0/3 | 2/3 |
| Von Oettingen et al. (1949)<br>Dogs, breed not specified,<br>5 females/group (control);<br>7 females/group (exposed)<br>90–97% pure, with 3–10% HMX; particle size not specified<br>0 or 50 mg/kg-d<br>Diet<br>6 d/wk for 6 wks | Treated dogs exhibited convulsions, excitability, ataxia, and hyperactive reflexes[a]; no incidence data were reported. | | | | |
| MacPhail et al. (1985)<br>Rats, Sprague-Dawley derived CD,<br>8–10 males or females/group<br>Purity 84 ± 4.7%; ≤66 µm particle size<br>0, 1, 3, or 10 mg/kg-d<br>Gavage<br>30 d | No changes in motor activity, flavor aversion, scheduled-controlled response, or acoustic startle-response were reported. | | | | |
| Cholakis et al. (1980)<br>Rats, F344, 24–25 females/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 6–19 | Doses | 0 | 0.2 | 2.0 | 20 |
| | **Convulsions** | | | | |
| | F | 0/24 | 0/24 | 1/24 | 18/25 |
| Angerhofer et al. (1986) (range-finding study)<br>Rats, Sprague-Dawley, 6 pregnant females/group<br>Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants<br>0, 10, 20, 40, 80, or 120 mg/kg-d<br>Gavage<br>GDs 6–15 | Convulsions preceding death were observed at ≥40 mg/kg-d; no incidence data were reported. | | | | |

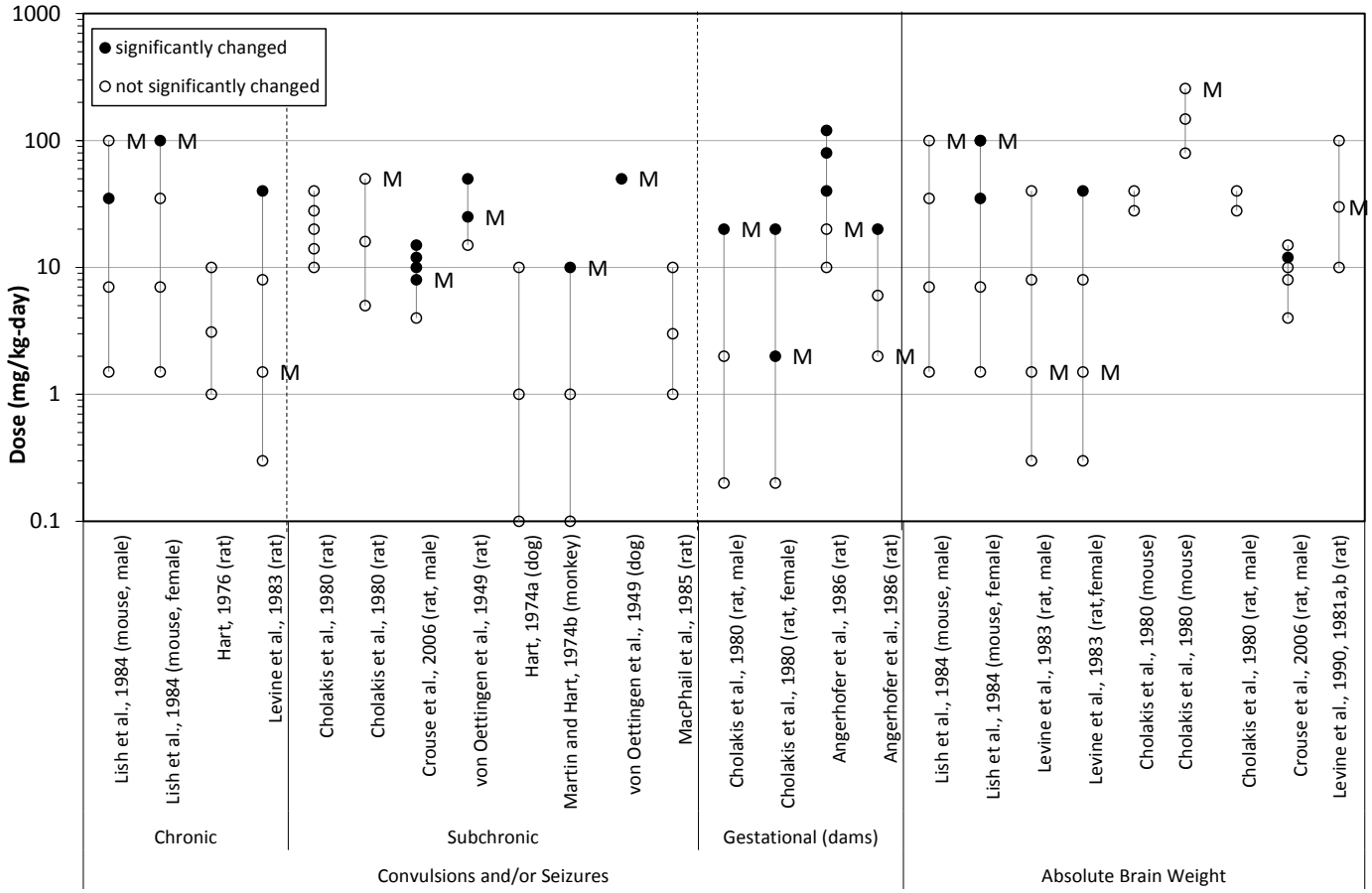
| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Angerhofer et al. (1986)<br>Rats, Sprague-Dawley, 39–51 mated females/group<br>Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants<br>0, 2, 6, or 20 mg/kg-d<br>Gavage<br>GDs 6–15 | Convulsions and hyperactivity[a] were observed at 20 mg/kg-day; no incidence data were reported. | | | | | |
| *Brain weight* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7 | 35 | 175/100 |
| | **Absolute brain weight** | | | | | |
| | M | 0% | −0.2% | 0.61% | 0.81% | −1% |
| | F | 0% | −2% | −2% | −4%* | −3%* |
| | **Relative brain weight** | | | | | |
| | M | 0% | 4% | 2% | 2% | 5% |
| | F | 0% | −4% | −1% | −3% | 18%* |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8 | 40 |
| | **Absolute brain weight** | | | | | |
| | M | 0% | 2% | −1% | 2% | 2% |
| | F | 0% | −0.3% | −0.4% | 1% | 2%* |
| | **Relative brain weight** | | | | | |
| | M | 0% | 0% | 8% | 2% | 22%* |
| | F | 0% | −1% | 3% | 4% | 20%* |
| Cholakis et al. (1980)<br>Mice, B6C3F1, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>Experiment 1: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute brain weight** | | | | | | |
| | M | 0% | – | – | – | 2% | 2% |
| | F | 0% | – | – | – | 4% | 2% |
| | **Relative brain weight** | | | | | | |
| | M | 0% | – | – | – | 6% | 2% |
| | F | 0% | – | – | – | 0% | 3% |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Experiment 2: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[b]<br>Diet<br>13 wks | Doses | 0 | 80 | 160 | 320 | |
| | **Absolute brain weight** | | | | | |
| | M | 0% | 0% | 2% | 10% | |
| | F | 0% | 0% | 4% | 2% | |
| | **Relative brain weight** | | | | | |
| | M | 0% | −3% | 1% | 8% | |
| | F | 0% | 0% | 3% | −4% | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute brain weight** | | | | | | |
| | M | 0% | – | – | – | 3% | 0% |
| | F | 0% | – | – | – | 0% | 0% |
| | **Relative brain weight** | | | | | | |
| | M | 0% | – | – | – | 7%* | 10%* |
| | F | 0% | – | – | – | 5% | 6% |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Absolute brain weight** | | | | | | |
| | M | 0% | −1% | −0.3% | 2% | 5%* | 7%* |
| | F | 0% | −2% | 6% | 1% | 4% | 6% |
| | **Relative brain weight** | | | | | | |
| | M | 0% | 6% | 10% | 5% | 3% | 4% |
| | F | 0% | −2% | −2% | −12%* | −12%* | −15%* |
| Levine et al. (1981a); Levine et al. (1990); Levine et al. (1981b)[d]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Absolute brain weight** | | | | | | |
| | M | 0% | 1% | 0.53% | −6% | – | – |
| | F | 0% | −1% | 1% | 2% | – | – |
| | **Relative brain weight** | | | | | | |
| | M | 0% | 4% | 7% | 14% | – | – |
| | F | 0% | 0.3% | 2% | 5% | – | – |

[a]Mortality was reported in some RDX-treated groups in this study.
[b]Doses were calculated by the study authors.
[c]Discrepancies in the doses at which convulsions occurred were identified in the technical report. The nervous system effects reported in this table and in the corresponding exposure-response array are those provided in the results section of the technical report (Levine et al., 1981a) and in the published paper (Levine et al., 1990). In other sections of the technical report, the authors reported that hyperactivity to approach and convulsions were observed in rats receiving ≥30 mg/kg-day (abstract and executive summary), or that mortality was observed in

rats receiving 100 mg/kg-d and that hyperactivity to approach, tremors, and convulsions were observed in animals exposed to lethal doses (discussion).

[d]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.




M- Mortality observed at this dose and above

**Figure 1-1. Exposure response array of nervous system effects following oral exposure.[3]**

[3]Due to the severity of the endpoint for convulsions and/or seizures, a response in treated groups was determined to be significant (filled circles) in the exposure-response array where there was an observation of convulsions and/or seizures reported in the study.

***Mechanistic Evidence***

The few studies that have explored the MOA of RDX on the central nervous system have focused on potential impacts on neurotransmission. These studies suggest that the MOA for RDX-induced seizures and convulsions involves distribution to the brain (across the blood-brain barrier) and subsequent effects on neurotransmitters, including gamma amino butyric acid (GABA) and glutamate. The strongest mechanistic information for RDX neurotoxicity comes from documented interactions with the $GABA_A$ receptor. GABA is a major inhibitory neurotransmitter in the brain, and the $GABA_A$ receptor has been implicated in susceptibility to seizures (Galanopoulou, 2008). It is also a target of many anticonvulsant therapies (e.g., benzodiazepines, propofol, barbiturates) (Meldrum and Rogawski, 2007; Möhler, 2006). The affinity of RDX for the $GABA_A$ receptor provides biological plausibility for the association of seizures with exposure to RDX in both human case reports and experimental animal studies.

In research conducted by the U.S. Army Center for Health Promotion and Preventative Medicine, Williams et al. (2011) and Bannon et al. (2009) showed a correlation between blood and brain concentrations of RDX in rats that received a single oral dose of RDX (>98–99.5% purity) by gavage, which closely correlated with the time of seizure onset. RDX (75 mg/kg) was distributed to the brain in direct proportion to levels found in the blood, while time to seizure onset was reduced as RDX brain levels increased (Williams et al., 2011). Similarly, oral exposure to RDX (via a gel capsule: 3 or 18 mg/kg) resulted in quick absorption followed by transport to the brain and subsequent alterations in neurotransmission (Bannon et al., 2009).

Some other pro-convulsant agents with minimal direct toxicity to nerve cells, such as sarin and some organophosphate pesticides, are known to act through inhibition of acetylcholinesterase (AChE) activity (Mcdonough and Shih, 1997). Some of the clinical signs observed following RDX exposure are similar to the clinical signs associated with organophosphate pesticides and nerve agents (Crouse et al., 2006; Burdette et al., 1988; Barsotti and Crotti, 1949). However, the limited data available for RDX do not support AChE inhibition as a primary mechanism because: 1) common AChE-induced symptoms such as salivation and lacrimation have not always been observed (Williams et al., 2011); 2) blood and brain levels of AChE are unaffected by RDX (Williams et al., 2011; Williams and Bannon, 2009); and 3) in vitro neurotransmitter receptor binding studies do not reveal any affinity of RDX for acetylcholine receptors (Williams et al., 2011; Williams and Bannon, 2009). RDX showed no affinity for other receptors that are known targets of convulsants, including the glutamate family of receptors, nicotinic receptors, glycine receptors, and several monoamine receptors (Williams et al., 2011; Williams and Bannon, 2009).

As noted above, in receptor binding assays RDX only showed affinity for $GABA_A$ receptors (Williams et al., 2011; Williams and Bannon, 2009). Specifically, RDX showed a significant affinity for the picrotoxin convulsant site of the GABA channel. The authors demonstrated that RDX treatment in brain slices from the basolateral amygdala inhibit $GABA_A$–mediated inhibitory postsynaptic currents and initiated seizure-like neuronal discharges. RDX exposure may reduce the

inhibitory effects of GABAergic neurons, resulting in enhanced excitability that could lead to seizures (Williams et al., 2011; Williams and Bannon, 2009), although additional studies are necessary to substantiate this observation and to clarify the potential cellular and regional targets of RDX-induced neurotoxicity.

The limbic system, and the amygdala and hippocampus in particular, are known to be critical to the development of seizures in various human conditions (e.g., epilepsy) and animal models (e.g., kindling) (Jefferys et al., 2012; Gilbert, 1994). Burdette et al. (1988) hypothesized that the limbic system was involved in seizures caused by RDX exposure, given than rats exhibited pro-convulsant activity in response to amygdaloid kindling at a dose that was approximately half the dose necessary for RDX to induce spontaneous seizures. Potential limbic system involvement is also suggested given its role in integrating emotional and behavioral responses (including aggression) and the anecdotal observations of hyperactivity, hyper-responsiveness, and irritability noted across several studies of RDX toxicity (Levine et al., 1990; Levine et al., 1983; Thompson, 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Von Oettingen et al., 1949).

In a microarray experiment, Bannon et al. (2009) found that RDX caused a down regulation of an abundance of genes in the cerebral cortex related to neurotransmission, including those encoding proteins involved in synaptic transmission and vesicle transport. Genes encoding proteins involved in the glutamate pathway were also underexpressed, indicating a possible mechanism of RDX via excessive glutamate stimulation. The authors speculated that this depression of the major excitatory neurotransmitter system could be a negative response to the increase in seizure likelihood from RDX influx into the brain. Molecular changes in response to RDX have been described by Zhang and Pan (2009b), who observed significant changes in micro-RNA (miRNA) expression in the brains of $B6C3F_1$ mice fed 5 mg/kg-day for 28 days. One miRNA, miR-206, was upregulated 26-fold in RDX-exposed brains; brain-derived neurotrophic factor (BDNF) was identified as a downstream gene target of this miRNA, along with two other miRNAs that were upregulated in RDX-exposed brains (miR-30a and miR-195) (Zhang and Pan, 2009a, b). BDNF is a member of the neurotrophin family of growth factors, and promotes the survival and differentiation of existing and new neurons. Effects of RDX on BDNF expression may play a role in RDX neurotoxicity, but the utility of miRNAs as predictors of toxicity has not been established, and the contribution, if any, of aberrant expression of a suite of miRNAs to the MOA for RDX neurotoxicity is unknown.

Information from a small number of studies suggests that inhibition of GABAergic signaling in the limbic system could represent a likely mechanism for RDX-induced hyperactivity and seizures. However, the available data are insufficient to identify any specific mode(s) of action for the nervous system effects observed following RDX exposure.

### *Summary of Nervous System Effects*

Evidence for nervous system effects associated with exposure to RDX comes from studies in both humans and animals. One occupational study reported memory impairment and decrements

in certain neurobehavioral tests in workers exposed to RDX compared to controls (Ma and Li, 1992), and human case reports provide other evidence of an association between acute RDX exposure and neurological effects. Eleven of 16 repeat-dose animal studies reported neurological effects, including seizures, convulsions, tremors, hyperirritability, hyper-reactivity and behavioral changes, associated with RDX exposure (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981b; Cholakis et al., 1980; Von Oettingen et al., 1949). In most of these studies, the occurrence of neurological effects was dose related. In those studies that found no evidence of RDX-associated neurotoxicity (MacPhail et al., 1985; Cholakis et al., 1980; Hart, 1976, 1974), differences in particle size and purity of the RDX administered could possibly account for the lack of effect. Although the specific mode(s) of action for RDX-induced nervous system effects remains unknown, evidence that RDX exposures may lead to seizures through binding to the $GABA_A$ receptor provides biological support for this association. EPA identified nervous system effects as a human hazard of RDX exposure.

### 1.1.2. Kidney and Other Urogenital System Effects

The association between RDX exposure and effects on clinical measures of kidney function was examined in a single occupational epidemiology study. Case reports involving accidental exposure to ingested or inhaled RDX provide some information on the potential for acute exposures to RDX to affect the kidney in humans. Organ weight and histopathology findings from experimental animal studies involving subchronic and chronic exposure to ingested RDX also provide data relevant to an examination of the association between RDX exposure and kidney and other urogenital system effects. A summary of kidney and other urogenital effects associated with RDX exposure is presented in Tables 1-3 to 1-7 and Figure 1-2.

Human case reports of individuals accidently exposed to unknown amounts of RDX by ingestion or inhalation provide some evidence that RDX may affect the kidney and urogenital system. Reported symptoms included decreased urine output (Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Merrill, 1968), blood in urine (Kasuske et al., 2009; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Merrill, 1968), proteinuria (Kasuske et al., 2009; Küçükardali et al., 2003; Ketel and Hughes, 1972; Hollander and Colbach, 1969; Merrill, 1968), glucosuria (Küçükardali et al., 2003), elevated blood urea nitrogen (BUN) levels (Hollander and Colbach, 1969; Merrill, 1968), and one case of acute renal failure requiring hemodialysis following accidental inhalation of RDX (Ketel and Hughes, 1972). In many of these case reports, renal parameters returned to normal within a few days following exposure. No changes in renal parameters were reported in other individuals exposed to unknown amounts of RDX (Stone et al., 1969; Kaplan et al., 1965). In a cross-sectional epidemiologic study of workers from five U.S. Army munitions plants (69 exposed to RDX alone and 24 to RDX and HMX; average exposure of up to 1.5 $mg/m^3$), no statistically significant differences in BUN or total serum protein between nonexposed and RDX-exposed groups were observed (Hathaway and Buck, 1977) (Table 1-3).

Studies in experimental animals provide some evidence that RDX exposure is associated with kidney and other urogenital effects (Table 1-4 and Figure 1-2). Dose-related increases in absolute and relative kidney weights (19–27% compared to control) were observed in male B6C3F$_1$ mice exposed to RDX in the diet for 2 years (Lish et al., 1984) and a dose-related increase in relative kidney weights (up to 19%) was observed in female mice. Relative, but not absolute, kidney weights were increased (20–21% compared to control) in male and female F344 rats exposed to 40 mg/kg-day RDX in the diet for 2 years (Levine et al., 1983). Changes in kidney weights in other subchronic oral toxicity studies in rats, dogs, and monkeys did not show a clear pattern of increase or decrease associated with RDX exposure; kidney weight changes were either not dose-related or were inconsistent across sexes when absolute and relative weights were compared (Crouse et al., 2008; Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Hart, 1974; Martin and Hart, 1974). Based on an evaluation of the relationship between organ weight and body/brain weight to determine which endpoint (organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio) is likely to more accurately detect target organ toxicity, Bailey et al. (2004) concluded that kidney weights are not modeled well by any of the choices, and that alternative analysis methods should be utilized.

Histopathological changes in the urogenital system associated with exposure to RDX were observed in a 2-year bioassay in which increased incidences of kidney medullary papillary necrosis and pyelitis, uremic mineralization, bladder distention and/or cystitis, and suppurative prostatitis were observed in high-dose (40 mg/kg-day) male rats that died spontaneously or were sacrificed in moribund condition (Levine et al., 1983). Similar kidney lesions were not observed in female rats in this study. An increased incidence of tubular nephrosis was observed in male B6C3F$_1$ mice exposed to 320 mg/kg-day RDX in feed for 90 days, but not in female mice in this study (Cholakis et al., 1980). In other chronic and subchronic oral studies in rats and mice, no histopathological changes in the kidney were associated with RDX exposure (Crouse et al., 2006; Levine et al., 1990; Lish et al., 1984; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Hart, 1976). Increased incidence of minimal to mild mineralization of the medulla was observed in male and female monkeys exposed to 10 mg/kg-day RDX for 90 days by gavage (Martin and Hart, 1974), but the study authors did not identify this as treatment related. No dose-related histopathological changes were reported in a subchronic study in dogs (Hart, 1974), and no histological alterations were noted in the kidneys of rabbits exposed dermally to 165 mg/kg RDX in DMSO for 4 weeks (McNamara et al., 1974). Measurement of serum chemistry parameters that may indicate effects on renal function, including BUN and uric acid, in studies of RDX in mice, rats, dogs, and monkeys (Crouse et al., 2008; Levine et al., 1990; Lish et al., 1984; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Hart, 1976, 1974; Martin and Hart, 1974) revealed variations (increases or decreases) from the respective control groups that were not dose-related.

Exposure to the major contaminant in many of the available RDX studies, HMX, was associated with histopathological changes in the kidney and alterations in renal function in female

rats fed doses ≥450 mg/kg-day HMX for 13 weeks. No effects were observed at doses ≤115 mg/kg-day. Because the percentage of HMX as an impurity ranged from 3–10% resulting in HMX exposures of ≤60 mg/kg-day in the studies of RDX toxicity, the contribution of HMX to the observed kidney toxicity in studies of RDX is expected to be negligible.

A significant, dose-related increase in the total incidence of suppurative prostatitis was reported in male F344 rats exposed to ≥1.5 mg/kg-day RDX in the diet for two years (Levine et al., 1983). The Levine et al. (1983) report is the only 2-year study that reported examination of the prostate in rats. Suppurative prostatitis was not observed in 90-day studies in the rat involving oral (dietary or gavage) exposure to RDX (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b). Similarly, prostate effects were not observed in a 2-year dietary study in mice (Lish et al., 1984). Some reports have hypothesized that the observation of prostate inflammation in Levine et al. (1983) is secondary to a bacterial infection unrelated to RDX toxicity (ATSDR, 2012; Sweeney et al., 2012a; Crouse et al., 2006). For example, Crouse et al. (2006) concluded that the inflammation reflects a common condition in rodents, noting that since 85% of the incidence occurred in rats found at spontaneous death or moribund sacrifice (SDMS), it was most likely that the condition was a result of an incidental bacterial infection. However, Levine et al. (1983) distinguish between nonsuppurative and suppurative inflammation (the latter being characterized by the formation of pus and a high concentration of neutrophils). Although the proportion of suppurative prostatitis was higher in SDMS rats, there was an increasing trend with dose in both the scheduled sacrifice (SS) and SDMS groups; the incidence of suppurative prostatitis in the control group was 4% when the SS and SDMS groups are combined. Additionally, the dose-related nature of the increased incidence suggests that the primary cause (potentially leading to bacterial infection) was treatment-related since a more uniform distribution of rats with suppurative prostatitis would be expected with a spontaneous or age-related lesion. The dose-responsiveness could be explained if the infections were secondary to treatment-related immunotoxicity, but there is no evidence from Levine et al. (1983) to support this possibility; a more thorough analysis of immune endpoints in a 90-day gavage exposure of F344 rats did not identify any immunotoxic effects associated with RDX (Crouse et al., 2006).

Levine et al. (1983) document an array of kidney and other urogenital lesions in their 2-year dietary exposure of F344 rats to RDX. However, the sequence by which those effects may have occurred is unclear. Renal medullary necrosis, bladder distension and cystitis were observed mainly in the male rats exposed to 40 mg/kg-day RDX for 24 months, although one rat in the 0.3 mg/kg-day dose group also exhibited these lesions. Treatment-related effects on the kidney (necrosis) and bladder (distension/obstruction and hemorrhagic cystitis) were also identified in the 12-month pathology report (see Tables 1-5 to 1-7). The absence of these observations in the 6-month interim pathology report suggests that an exposure duration of greater than 6 months may be required before RDX-induced effects on the urogenital system are observed. Suppurative prostatitis was observed with increasing incidence in each dose group in the study at 24 months.

Considered as a group, treatment-related kidney and urogenital lesions may have led to a blockage that resulted in urinary stasis. Reduced urinary flow and/or retrograde flow may have contributed to an environment that allowed bacterial infection of the prostate. Thus while an opportunistic bacterial infection could be the proximal cause of the suppurative prostatitis, it may have been secondary to the effects of RDX on the urogenital system. This hypothesis is consistent with the observed dose-related increase in incidence of the suppurative prostatitis (ATSDR, 2012; Sweeney et al., 2012a; Crouse et al., 2006).

Although the ultimate sequence of effects in the urogenital system is unclear, even from review of the scheduled sacrifices at 6 or 12 months on study, it is plausible that the observations of suppurative prostatitis would arise after other kidney or bladder lesions that resulted in the initial blockage and urinary stasis. The incidence of suppurative prostatitis reported in Levine et al. (1983) was increased at doses lower than the doses associated with an increased incidence of other urogenital lesions. However, the incidence of bladder lesions may have been underreported, since the bladders were only examined following observation of a gross abnormality. Bladder distension was reported sporadically among the lower dose groups (0.3, 1.5, or 8.0 mg/kg-day), but the bladder was not routinely examined in these dose groups (Levine et al., 1983; Thompson, 1983). Although the pathogenesis of kidney and urogenital effects cannot be established, the available evidence is consistent with suppurative prostatitis as an indirect effect of RDX exposure and as a marker for the broader array of kidney and urogenital effects observed by Levine et al. (1983).

**Table 1-3. Evidence pertaining to kidney effects in humans**

| Reference and study design | Results | | | |
|---|---|---|---|---|
| Hathaway and Buck (1977)<br>Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate).<br>Analysis group: limited to whites; 69 workers exposed to RDX alone and 24 workers exposed to RDX and HMX, compared to 338 workers not exposed to RDX, HMX, or TNT.<br>**Exposure measures**: Exposure determination based on job title and industrial hygiene evaluation; exposed subjects assigned to two groups: undetected (<LOD) or ≥0.01 mg/m$^3$ (mean 0.28 mg/m$^3$).<br>**Effect measures**: Renal function tests (blood)<br>**Analysis**: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and $\chi^2$ tests for comparison of proportions). | **Renal function tests in men: mean** *(standard deviation not reported)* | | | |
| | | RDX exposed | | |
| | Test | Referent (n = 237) | Undetected (n = 22) | >0.01 mg/m$^3$ (n = 45) |
| | BUN | 15.5 | 15.6 | 16.4 |
| | Total protein | 7.2 | 7.2 | 7.3 |
| | No differences were statistically significant. Similar results in women. | | | |

**Table 1-4. Evidence pertaining to kidney and other urogenital system effects in animals**

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| *Kidney weight* | | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 | |
| | **Absolute kidney weight at 104 wks** *(percent change compared to control)* | | | | | | |
| | M | 0% | −1% | 4% | 9%* | 19%* | |
| | F | 0% | 3% | 1% | 1% | −2% | |
| | **Relative kidney weight at 104 wks** *(percent change compared to control)* | | | | | | |
| | M | 0% | 3% | 6% | 11%* | 27%* | |
| | F | 0% | 1% | 1% | 2% | 19%* | |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | 10 | | |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −3% | −7% | 2% | | |
| | F | 0% | 14% | −4% | 8% | | |
| | **Relative kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −1% | −4% | 4% | | |
| | F | 0% | 22% | 3% | 18% | | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 | |
| | **Absolute kidney weight at 105 wks** *(percent change compared to control)* | | | | | | |
| | M | 0% | 2% | −7% | 1% | 0% | |
| | F | 0% | 3% | 3% | 2% | 2% | |
| | **Relative kidney weight at 105 wks** *(percent change compared to control)* | | | | | | |
| | M | 0% | 1% | 0% | 2% | 20%* | |
| | F | 0% | 3% | 6% | 5% | 21%* | |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>**Experiment 1**: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 18% | 2% |
| | F | 0% | – | – | – | −8% | −5% |
| | **Relative kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 29% | 0% |
| | F | 0% | – | – | – | −8% | −3% |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| **Experiment 2**: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a]<br>Diet<br>13 wks | Doses | 0 | 80 | 160 | 320 | | |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 8% | 11% | 13% | | |
| | F | 0% | −5% | −3% | 0% | | |
| | **Relative kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 5% | 9% | 10% | | |
| | F | 0% | −5% | −4% | −5% | | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −2% | −5% |
| | F | 0% | – | – | – | 1% | 0% |
| | **Relative kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 1% | 5% |
| | F | 0% | – | – | – | 6% | 6% |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study;<br>F0: 22/sex/group; F1: 26/sex/group;<br>F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, 50 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 5 | 16 | 50 | | |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 6% | −12% | – | | |
| | F | 0% | −4% | −21%* | – | | |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −3% | −4% | −1% | 3% | 5% |
| | F | 0% | 2% | 5% | 13%* | 10% | 15%* |
| | **Relative kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 3% | 6% | 2% | 1% | 3% |
| | F | 0% | 1% | −3% | −1% | −6% | −7%* |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[b]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d | Data were not reported for rats in the 300 or 600 mg/kg-d groups because all of the rats died before the 13-wk necropsy. | | | | | | |
| | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 1% | 1% | −9% | – | – |
| | F | 0% | 1% | 3% | −1% | – | – |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Diet<br>13 wks | **Relative kidney weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 5% | 7% | 10% | – | – |
| | F | 0% | 3% | 5% | 2% | – | – |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Numerical values given only for control and 10 mg/kg-d groups. | | | | | |
| | Doses | 0 | | 0.1 | 1 | 10 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | |
| | M | 0% | | – | – | 38% |
| | F | 0% | | – | – | –18% |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | | 0.1 | 1 | 10 |
| | **Absolute kidney weight** *(percent change compared to control)* | | | | | |
| | M + F | 0% | | –2% | –3% | 4% |
| *Histopathological lesions* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | The incidence of cytoplasmic vacuolization of renal tubules was greater for RDX-treated males than the control group males after 6 mo of treatment. However, at 12 and 24 mo of treatment, this lesion was observed as frequently in control animals as animals treated with RDX. | | | | | |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Histopathological examination of kidney did not reveal any significant differences compared to controls; lesions observed were not attributed to RDX treatment; incidence data were reported only for control and 10 mg/kg-d groups. | | | | | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo<br>Note: More detailed histopathological results, including interim sacrifice data at 6 and 12 mo, are provided in Tables 1-5 to 1-7. | Data were analyzed separately for animals sacrificed on schedule (SS) and those that died spontaneously or were sacrificed moribund (SDMS); incidence data were not reported for females. | | | | | |
| | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Kidney, medullary papillary necrosis; 24 mo** *(incidence)* | | | | | |
| | (SS) | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| | (SDMS) | 0/17 | 1/19 | 0/27 | 0/26 | 18/27* |
| | (Sum) | 0/55 | 1/55 | 0/52 | 0/55 | 18/31* |
| | **Kidney, suppurative pyelitis; 24 mo** *(incidence)* | | | | | |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| | (SS) | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| | (SDMS) | 0/17 | 1/19 | 0/27 | 1/26 | 5/27* |
| | (Sum) | 0/55 | 1/55 | 0/52 | 1/55 | 5/31* |
| | **Kidney, uremic mineralization; 24 mo** *(incidence)* | | | | | |
| | (SS) | 1/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| | (SDMS) | 0/17 | 1/19 | 2/27 | 0/26 | 13/27 |
| | (Sum) | 1/55 | 1/55 | 2/52 | 0/55 | 13/31 |
| | **Urinary bladder, luminal distention; 24 mo** *(incidence)* | | | | | |
| | (SS) | 0/38 | 0/36 | 0/25 | 0/29 | 1/4* |
| | (SDMS) | 0/16 | 2/19 | 1/27 | 3/22 | 24/28* |
| | (Sum) | 0/54 | 2/55 | 1/52 | 3/51 | 25/32* |
| | **Urinary bladder, cystitis hemorrhagic/suppurative; 24 mo** *(incidence)* | | | | | |
| | (SS) | 0/38 | 0/36 | 0/25 | 1/29 | 0/4 |
| | (SDMS) | 0/16 | 2/19 | 1/27 | 0/22 | 18/27* |
| | (Sum) | 0/54 | 2/55 | 1/52 | 1/51 | 18/31* |
| | **Prostate, suppurative inflammation (prostatitis); 24 mo** *(incidence)* | | | | | |
| | SS | 0/38 | 1/36 | 2/25* | 4/29* | 0/4 |
| | SDMS | 2/16 | 3/19 | 7/27* | 8/26 | 19/27* |
| | (Sum) | 2/54 | 4/55 | 9/52* | 12/55* | 19/31* |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10−12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 80, 60, 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a]<br>Diet<br>13 wks | Incidence data reported only for controls and the 320 mg/kg-d group. | | | | | |
| | Doses | 0 | 80 | 160 | 320 | |
| | **Tubular nephrosis** *(incidence)* | | | | | |
| | M | 0/10 | – | – | 4/9* | |
| | F | 0/11 | – | – | 1/11 | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Histopathological examination of kidney did not reveal any significant differences compared to controls; incidence data were reported only for control and 40 mg/kg-d groups. | | | | | |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Cholakis et al. (1980)<br>Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | Data were reported only for F2 generation controls and 5 and 16 mg/kg-d groups. | | | | | |
| | Doses | 0 | 5 | 16 | 50 | |
| | **Cortical cysts (incidence)** | | | | | |
| | M | 4/10 | 4/10 | 8/10 | – | |
| | F | 3/10 | 4/10 | 8/10 | – | |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Prostate, mild subacute inflammation** *(incidence)* | | | | | | |
| | M | 0/10 | – | – | – | – | 1/8 |
| | Histopathological examination of kidney did not reveal any significant differences compared to controls; incidence data were reported only for control and 15 mg/kg-d groups. | | | | | | |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[b]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Histopathological examination of kidney did not reveal any significant differences compared to controls. | | | | | |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Histopathological examination of kidney did not reveal any significant differences compared to controls; incidences were reported only for control and 10 mg/kg-d groups. | | | | | |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 0.1 | 1 | 10 | |
| | **Medulla; mineralization, minimal to mild** *(incidence)* | | | | | |
| | M + F | 0/6 | 1/6 | 0/6 | 4/6 | |

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.

**Table 1-5. Six-, 12-, and 24-month incidence of kidney endpoints in male F344 rats reported for statistical evaluation in Levine et al. (1983)**

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| **Medullary papillary necrosis** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 15/19* |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 15/29* |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| SDMS | 0/17 | 1/19 | 0/27 | 0/26 | 18/27* |
| Sum | 0/55 | 1/55 | 0/52 | 0/55 | 18/31* |
| **Pyelitis** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 1/19 |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 1/29 |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| SDMS | 0/17 | 1/19 | 0/27 | 1/26 | 5/27* |
| Sum | 0/55 | 1/55 | 0/52 | 1/55 | 5/31* |
| **Pyelonephritis** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 1/19 |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 1/29 |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 1/29 | 0/4 |
| SDMS | 0/17 | 0/19 | 2/27 | 1/26 | 1/27 |
| Sum | 0/55 | 0/55 | 2/52 | 2/55 | 1/31 |

*Statistically significant ($p < 0.05$) based on analysis by study authors.

Source: Levine et al. (1983).

**Table 1-6. Six-, 12-, and 24-month incidence of urinary bladder endpoints in male F344 rats reported for statistical evaluation in Levine et al. (1983)**

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| **Luminal distention** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 18/19* |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 18/29 |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 0/29 | 1/4* |
| SDMS | 0/16 | 2/19 | 1/27 | 3/22 | 24/28* |
| Sum | 0/54 | 2/55 | 1/52 | 3/51 | 25/32* |
| **Cystitis, hemorrhagic/suppurative** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 17/19* |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 17/29 |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 1/29 | 0/4 |
| SDMS | 0/16 | 2/19 | 1/27 | 0/22 | 18/27* |
| Sum | 0/54 | 2/55 | 1/52 | 1/51 | 18/31* |

*Statistically significant ($p < 0.05$) based on analysis by study authors.

Source: Levine et al. (1983).

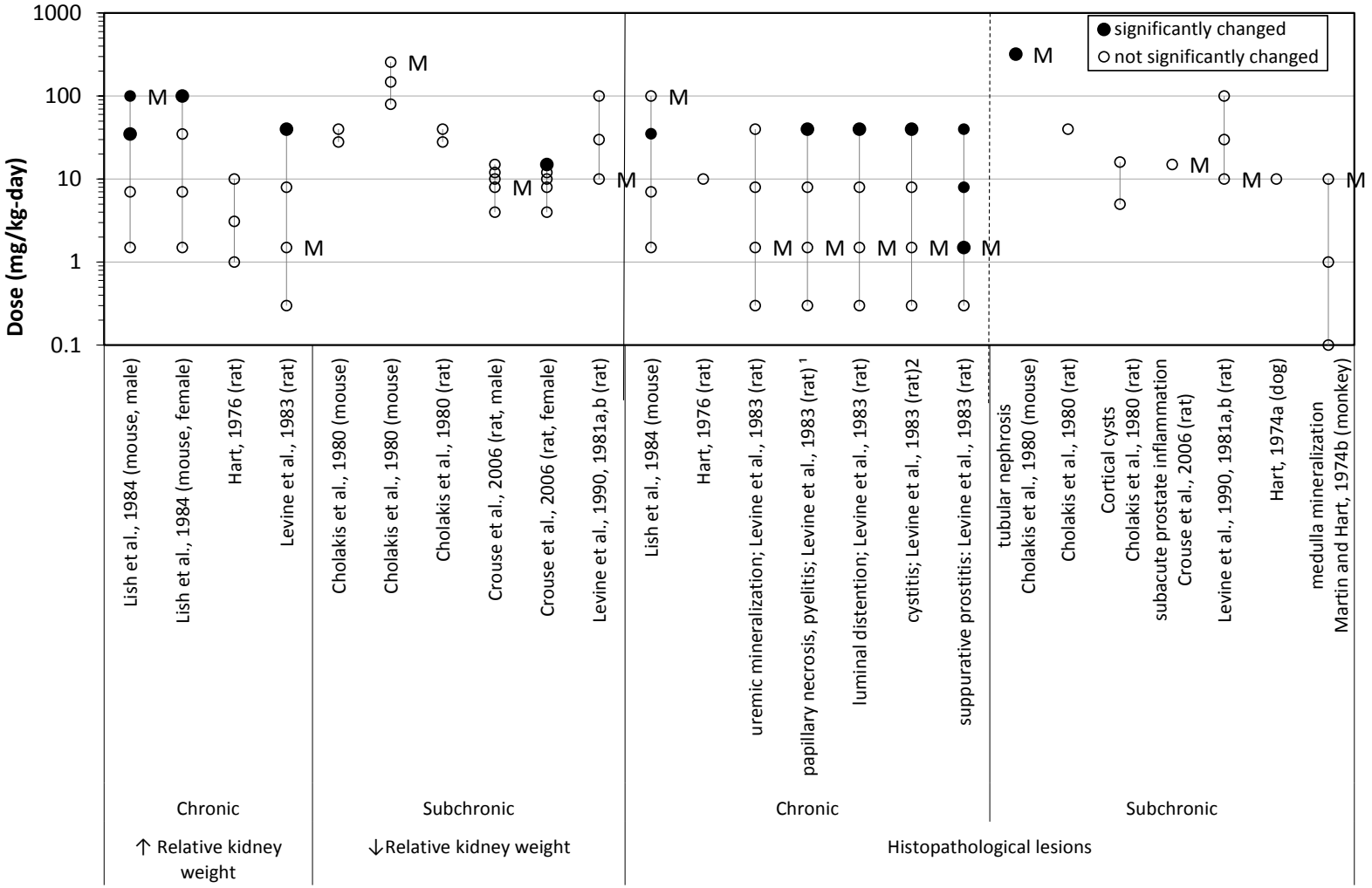
**Table 1-7. Six-, 12-, and 24-month incidence of prostate endpoints in male F344 rats reported for statistical evaluation in Levine et al. (1983)**

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| **Spermatic granuloma** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 2/10 | 2/10 | 1/10 | 6/10* |
| SDMS | - | - | - | - | 2/5 |
| Sum | 0/10 | 2/10 | 2/10 | 1/10 | 8/15* |
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 1/10 | 1/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 0/19 |
| Sum | 0/10 | 0/10 | 1/13 | 1/10 | 0/29 |
| 24 mo | | | | | |
| SS | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| SDMS | 0/16 | 0/19 | 0/27 | 0/26 | 0/27 |
| Sum | 0/54 | 0/55 | 0/52 | 0/55 | 0/31 |
| **Suppurative inflammation** *(incidence)* | | | | | |
| 6 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | - | - | 0/5 |
| Sum | 0/10 | 0/10 | 0/10 | 0/10 | 0/15 |

| Doses (mg/kg-d) | 0 | 0.3 | 1.5 | 8.0 | 40 |
|---|---|---|---|---|---|
| 12 mo | | | | | |
| SS | 0/10 | 0/10 | 0/10 | 0/10 | 0/10 |
| SDMS | - | - | 0/3 | - | 0/19 |
| Sum | 0/10 | 0/10 | 0/13 | 0/10 | 0/29 |
| 24 mo | | | | | |
| SS | 0/38 | 1/36 | 2/25* | 4/29* | 0/4 |
| SDMS | 2/16 | 3/19 | 7/27* | 8/26 | 19/27* |
| Sum | 2/54 | 4/55 | 9/52* | 12/55* | 19/31* |

*Statistically significant ($p < 0.05$) based on analysis by study authors.

Source: Levine et al. (1983).




The following studies were excluded from array because absolute kidney weight was reported: Cholakis, 1980 (2-gen rat); Hart, 1974; Martin and Hart, 1974
M - Mortality observed at this dose and above
[1] statistical significance determined from incidence at time of of scheduled sacrfice
[2] statistical significance determined from incidence at spontaneous death.

**Figure 1-2. Exposure-response array of kidney and urogenital system effects.**

***Mechanistic Evidence***

No MOA information is available for RDX-induced kidney and other urogenital effects, including suppurative prostatitis. However, mechanistic information underlying the neurotoxicity observed with RDX exposure, and the specific affinity of RDX to the $GABA_A$ receptor-convulsant site (Williams et al., 2011; Williams and Bannon, 2009), suggests a biologically plausible role for the $GABA_A$ receptor in RDX-related effects on the urogenital system and provides some potential modes of action for the effects reported in Levine et al. (1983).

Alterations in hormonal signaling or circulating levels of estrogen or prolactin may lead to prostatitis. Prostate inflammation has been associated with endocrine disruptors in the environment (Cowin et al., 2010), and increased prolactin has been shown to cause lateral lobe prostatitis (Stoker et al., 1999b; Stoker et al., 1999a; Tangbanluekal and Robinette, 1993; Robinette, 1988). Typically the inflammation seen is chronic and does not reverse over time (Robinette, 1988). Functional $GABA_A$ receptors have been identified in the anterior pituitary (Zemkova et al., 2008; Mayerhofer, 2001), which also serves as the primary source of prolactin. Thus, the prostate inflammation observed in the rat in the 2-year study by Levine et al. (1983) could have been produced by disruption of pituitary prolactin or other hormonal signal via interference with normal regulatory GABA-related hormonal control. However, no direct evidence for this hypothesized MOA is available. Levine et al. (1983) did not evaluate serum endocrine measures or pituitary weights, and pituitary adenomas that could account for higher prolactin levels were not observed. A MOA based on pituitary-mediated alterations in endocrine signaling also does not explain the other urogenital lesions observed by Levine et al. (1983).

Another hypothesis is that the prostate effects could be mediated through an autoimmune inflammatory response. $GABA_A$ receptor transcripts have been identified in immune cells of mouse models (Reyes-García et al., 2007; Tian et al., 2004), and $GABA_A$ receptor agonists have decreased cytotoxic immune responses and hypersensitivity reactions (Tian et al., 1999; Bergeret et al., 1998). In a murine autoimmune model of multiple sclerosis, Bhat et al. (2010) found that treatment of macrophages challenged with lipopolysaccharide with various GABA agonists decreased cytokine production; addition of picrotoxin (which may have effects similar to those of RDX, since they bind to the same site) was able to reduce this effect. However, picrotoxin on its own did not significantly alter cytokine production, suggesting the effects are limited to reversal of agonist-induced GABAergic activity. If an autoimmune mechanism was contributing to the effects observed with RDX exposure, it is unclear why inflammation would be limited to the prostate. RDX has also tested negative in the only battery of immunotoxicity tests to which it was subjected (Crouse et al., 2006).

If it is assumed that the kidney and other urogenital effects are mediated through localized interaction with $GABA_A$ receptors, another possibility is that effects would result from direct interactions with $GABA_A$ receptors located on the prostate. $GABA_A$ receptors have been identified on the prostate (Napoleone et al., 1990), providing a potential mechanism by which RDX could interact directly with the prostate. However, this would require that the prostate is actively

maintained in a non-inflamed state, mediated by GABA; RDX binding to $GABA_A$ receptor-convulsant sites on the prostate would result in a reduction of the inhibitory effects of the GABA receptor leading to increased inflammation. No evidence was found to support this potential pathway leading to prostate inflammation.

Another hypothesis is that the kidney and other urogenital effects of RDX are caused by interactions with $GABA_A$ receptors mediating inputs to the urogenital system. GABA is believed to play a role in the regulation of urination and bladder capacity (reviewed in Fowler et al. (2008) and Yoshimura and de Groat (1997)). In rats, injection of a $GABA_A$ receptor agonist inhibits the urination reflex (Igawa et al., 1993; Kontani et al., 1987). $GABA_A$ agonists injected into the periaqueductal gray area in rats inhibited reflex bladder activity, while injection of an antagonist reduced bladder capacity and increased the frequency of bladder reflex activity (Stone et al., 2011). RDX would be expected to act like an antagonist and increase bladder activity (which would not result in urinary stasis), although the impact of chronic exposure to RDX acting as a $GABA_A$ receptor antagonist is not known. Evidence of GABAergic signaling regulating bladder function, and the hypothesized disruption of that regulation by RDX via interaction with $GABA_A$ receptors, may plausibly account for the kidney and other urogenital lesions, including suppurative prostatitis, observed by Levine et al. (1983); however, no evidence to support this hypothesized MOA is available.

In summary, there are no studies available that inform mechanistically how RDX might lead to kidney and other urogenital effects. There is evidence that RDX binds to $GABA_A$ receptors in neuronal tissues (Williams et al., 2011; Williams and Bannon, 2009), and it is biologically plausible that binding to the GABA receptor could occur in other tissues as well, accounting for the observed kidney and urogenital effects. Among the mechanistic information presented above, modes of action that require direct action on the prostate are considered less likely, because the available information suggests the prostatitis is a secondary effect. However, the ways $GABA_A$ receptors work in non-neuronal tissues and organs is still not well understood, and the MOA by which RDX induces kidney and other urogenital effects is unknown.

### *Summary of Kidney and Other Urogenital System Effects*

Evidence for kidney effects resulting from RDX exposure consists of human case reports and some findings of increased kidney weight and histopathological changes in rodents. In humans, evidence for kidney effects (including decreased urine output, blood in urine, and proteinuria) is limited to individuals with acute accidental exposure (ingestion and inhalation) to unknown amounts of RDX. No RDX-related changes in kidney parameters were found in a small cross-sectional study of RDX-exposed workers (Hathaway and Buck, 1977). Treatment-related increases in relative kidney weight were consistently observed in rats and mice of both sexes in two chronic oral toxicity studies (Lish et al., 1984; Levine et al., 1983); however, kidney weights across studies of subchronic duration generally failed to show a consistent pattern of change. Measurement of

serum chemistry parameters in multiple animal species did not provide consistent evidence of dose-related changes associated with RDX exposure.

Histopathological changes in a two-year study in F344 rats, including a dose-related increase in the incidence of suppurative prostatitis in male rats (Levine et al., 1983; Thompson, 1983), provides the strongest evidence of RDX-associated kidney and other urogenital effects. As discussed above, the incidence of suppurative prostatitis is considered to be an indicator for the broader array of kidney and other urogenital effects seen in this study. A second 2-year study in Sprague-Dawley rats found no histopathological changes in the kidney or urogenital system (Hart, 1976), but exposure levels used in this study were low compared to Levine et al. (1983). In light of the dose-related increase in suppurative prostatitis and lack of support for an alternative (i.e., non-RDX-related) basis for this effect, EPA identified kidney and other urogenital effects as a potential human hazard of RDX exposure.

### 1.1.3. Reproductive and Developmental Effects

No human studies were identified that evaluate the potential of RDX to cause reproductive or developmental effects. Information relevant to an examination of the association between RDX exposure and reproductive and developmental effects comes from a 2-generation study in rats and studies in rats and rabbits involving gestational exposure to ingested RDX. In addition, oral subchronic and chronic studies in experimental animals provide information useful for examining the association between RDX exposure and effects on the male reproductive system. A summary of the developmental and reproductive effects associated with RDX exposure is presented in Tables 1-8 and 1-9 and Figures 1-3 and 1-4.

Developmental Effects

Animal studies report effects of RDX on offspring survival. Pup survival rates in the F0 and F1 generations were statistically significantly decreased in RDX-exposed CD rats compared to controls in the only available two-generation reproductive toxicity study of RDX (Cholakis et al., 1980), but only at the highest dose tested (50 mg/kg-day) that also produced toxicity in adults (neurotoxicity, mortality, and reduced body weights and food consumption). Decreased fetal viability was observed at 20 mg/kg-day in F344 rats (Cholakis et al., 1980), although no effect on live fetuses was observed in Sprague-Dawley rats at the same dose (Angerhofer et al., 1986); both of these studies reported significant mortality in dams at 20 mg/kg-day. Increased resorptions were similarly limited to the highest dose tested (20 mg/kg-day), i.e., a dose associated with maternal toxicity (Cholakis et al., 1980). There was no evidence of maternal toxicity, embryotoxicity or decreased fetal viability in a teratology study of pregnant rabbits exposed to RDX by gavage from GD 7 to 29 at doses up to 20 mg/kg-day (Cholakis et al., 1980), suggesting that rabbits may be less sensitive to RDX toxicity than rats.

Statistically significant, dose-related reductions in fetal body weight and length were reported in Sprague-Dawley rats exposed to RDX by gavage from GD 6 to 15 (Angerhofer et al.,

1986).[4] Maximum decreases in fetal body weight (9%) and body length (5%) were observed at 20 mg/kg-day, a dose that produced significant mortality in the dams. A similar reduction in fetal body weight of 7% (not statistically significant) was observed in F344 rats exposed to RDX at 20 mg/kg-day, a dose associated with maternal mortality (Cholakis et al., 1980). The larger Sprague-Dawley litter sizes and number of fetuses, compared to F344 rats, may account for the greater statistical power to observe treatment-related effects. Dose-related reductions in fetal body weight were not observed in rabbits at doses up to 20 mg/kg-day (Cholakis et al., 1980).

No treatment-related teratogenic effects have been reported in rats exposed to a dose as high as 20 mg/kg-day RDX, a dose that resulted in approximately 30% maternal mortality (Angerhofer et al., 1986; Cholakis et al., 1980). Examination of rabbits administered RDX at doses up to 20 mg/kg-day from GD 7 - 29 also provided little evidence of teratogenicity (Cholakis et al., 1980). Increased incidences of enlarged front fontanel and unossified sternebrae were observed in all groups of rabbits exposed to RDX (Cholakis et al., 1980); however, these developmental anomalies did not exhibit a dose-related increase. Gestational exposure to RDX did not result in any other skeletal abnormalities.

Reproductive Effects

Evidence of male reproductive toxicity is provided by the finding of testicular degeneration in male mice (Table 1-9 and Figure 1-4). An increased incidence of testicular degeneration was observed in male B6C3F$_1$ mice exposed to ≥35 mg/kg-day RDX for 2 years in the diet (10–11%) compared to concurrent (0%) and historical (1.5%) controls (Lish et al., 1984). Reductions in absolute testicular weight were observed, but the magnitude of the effect was small (≤6% compared to controls) and not dose-related. An increased incidence of germ cell degeneration was observed in rats exposed to 40 mg/kg-day (40%) compared with controls at 12 months (0%); by 24 months all male rats (including controls) had testicular masses and no instances of germ cell degeneration were identified in control or RDX-treated groups (Levine et al., 1983). No dose-related histopathological changes in the testes were identified in other studies in rats (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Hart, 1976) or dogs (Hart, 1974). Changes in testicular weight were inconsistent across studies, with an equivalent number of studies identifying decreases (Crouse et al., 2006; Lish et al., 1984; Cholakis et al., 1980) or increases (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Hart, 1976, 1974) in testicular weight; in most cases the changes in testicular weight were small (≤10% change compared to control) and not dose-related. Based on an evaluation of the relationship between organ weight and body/brain weight to determine which endpoint (organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio) is likely to more accurately detect target organ

[4] The statistical analyses presented by the study authors were performed on a per fetus basis; EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991) recommend that fetal data be analyzed on a per litter (rather than per fetus) basis. In a reanalysis of the Angerhofer et al. (1986) data by EPA on a per litter basis, fetal body weight and length showed statistically significant decreasing trends.

toxicity, Bailey et al. (2004) concluded that testes weights are not modeled well by any of the choices, and that alternative analysis methods should be utilized.

Reproductive function was assessed in two separate studies reported by Cholakis et al. (1980). In the dominant lethal mutation study, no effects on fertility were observed in male rats exposed to ≤16 mg/kg-day RDX. Pregnancy rates were lower in females mated to males exposed to 50 mg/kg-day RDX for 15 weeks prior to mating, although this effect was attributed to decreased well-being of the males in this high-dose group (Cholakis et al., 1980). No specific effects on reproductive function were observed in F0 and F1 rats exposed to ≤16 mg/kg-day RDX in a two-generation study. The highest dose tested, 50 mg/kg-day, was associated with reductions in fertility (specifically a decreased number of pregnancies) in the F0 generation, although these changes were not statistically significant. The finding of lower fertility rates only at the 50 mg/kg-day dose, a dose associated with reduced body weight and feed consumption and increased mortality, suggests that effects on reproductive function were likely due to the general toxicity of RDX rather than a direct effect of RDX on reproduction.

**Table 1-8. Evidence pertaining to reproductive and developmental effects in animals**

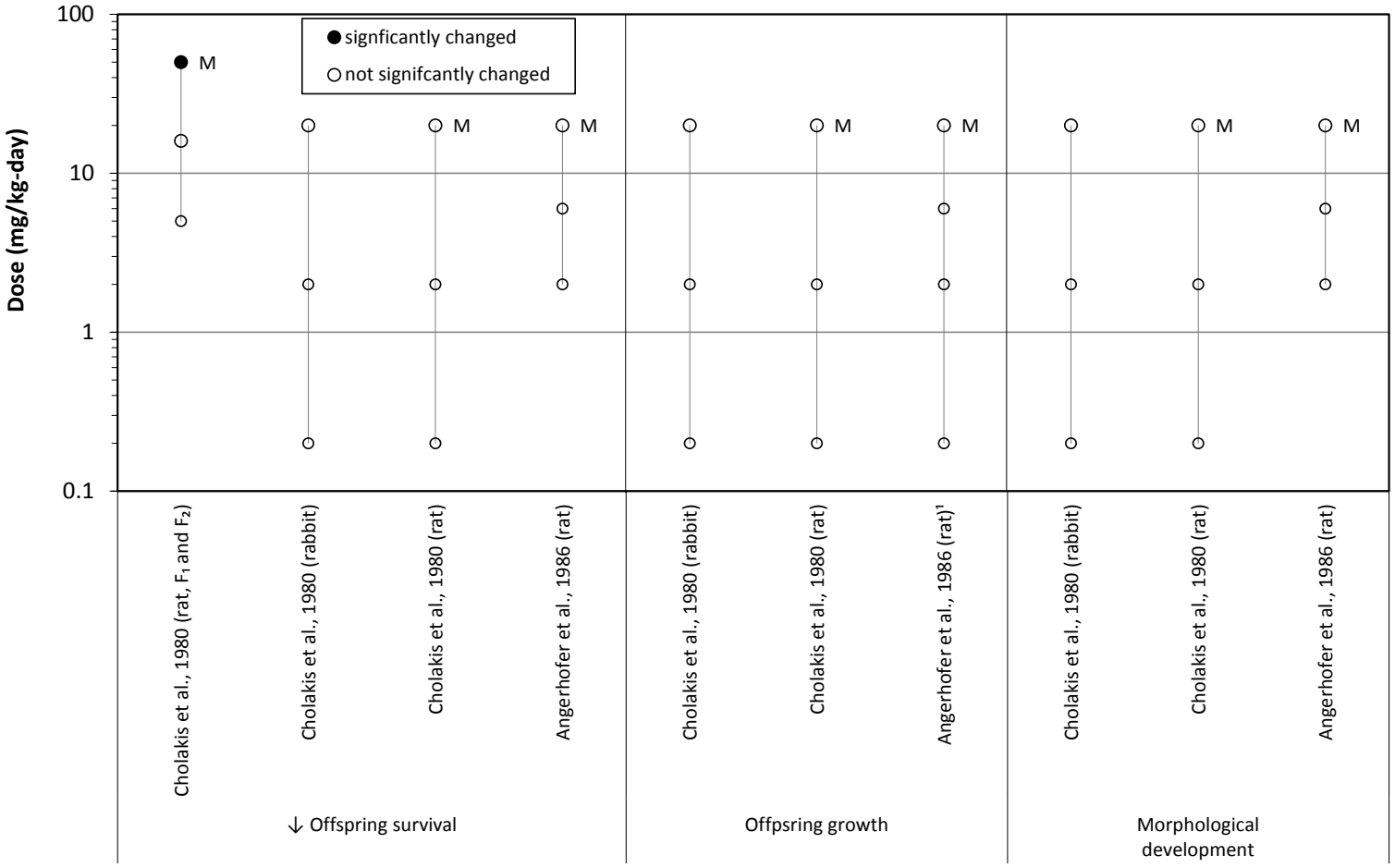
| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| *Offspring survival* | | | | | |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study;<br>F0: 22/sex/group; F1: 26 sex/group;<br>F2: 10 sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 5 | 16 | 50 |
| | **Stillborn pups** *(incidence)* | | | | |
| | F1 | 8/207 | 6/296 | 4/259 | 16/92* |
| | F2 | 6/288 | 6/290 | 2/250 | 24/46* |
| | **Offspring survival at birth** *(percent of fetuses)* | | | | |
| | F1 | 96% | 98% | 98% | 83%* |
| | F2 | 98% | 98% | 99% | 48%* |
| | F0 maternal deaths occurred at 50 mg/kg-d. Only six F1 females in this group survived to serve as parental animals; none of the six died during subsequent treatment.<br>Note: results on a per litter basis were not provided. | | | | |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| Cholakis et al. (1980)<br>Rabbits, New Zealand White, 11–12/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 7–29 | Doses | 0 | 0.2 | 2 | 20 |
| | **Early resorptions** *(mean percent per dam)* | | | | |
| | | 6% | 5% | 4% | 1% |
| | **Late resorptions** *(mean percent per dam)* | | | | |
| | | 8% | 5% | 3% | 3% |
| | **Complete litter resorptions** *(number of litters)* | | | | |
| | | 0 | 0 | 0 | 2 |
| | **Viable fetuses** *(mean percent per dam)* | | | | |
| | | 85% | 82% | 77% | 94% |
| Cholakis et al. (1980)<br>Rats, F344, 24–25 females/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants.<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 6–19 | Doses | 0 | 0.2 | 2.0 | 20 |
| | **Early resorptions** *(mean percent per dam)* | | | | |
| | | 6.0% | 2.5% | 4.8% | 15.3% |
| | **Late resorptions** *(mean percent per dam)* | | | | |
| | | 0.5% | 0.5% | 0.3% | 1.6% |
| | **Complete litter resorptions** *(number of litters)* | | | | |
| | | 0 | 0 | 0 | 2 |
| | **Viable fetuses** *(mean percent per dam)* | | | | |
| | | 93.2% | 97.6% | 94.9% | 81.4% |
| | Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d. | | | | |
| Angerhofer et al. (1986)<br>Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group)<br>Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants<br>0, 2, 6, or 20 mg/kg-d<br>Gavage<br>GDs 6–15 | Doses | 0 | 2 | 6 | 20 |
| | **Resorptions** *(percent of total implantations)* | | | | |
| | | 4.8% | 6.1% | 5.9% | 6.4% |
| | **Early resorptions** *(percent of total implantations)* | | | | |
| | | 4.8% | 6.1% | 5.9% | 6.2% |
| | **Late resorptions** *(percent of total implantations)* | | | | |
| | | 0% | 0% | 0% | 0.27% |
| | **Live fetuses** *(mean percent per litter)* | | | | |
| | | 100% | 100% | 100% | 100% |
| | Significant maternal mortality (16/51) occurred at 20 mg/kg-d. | | | | |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| *Offspring growth* | | | | | |
| Cholakis et al. (1980)<br>Rabbits, New Zealand White, 11–12/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 7–29 | Doses | 0 | 0.2 | 2.0 | 20 |
| | **Fetal body weight** *(percent change compared to control)* | | | | |
| | | 0% | −6.7% | −2.3% | −9.3% |
| Cholakis et al. (1980)<br>Rats, F344, 24–25 females/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants.<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 6–19 | Doses | 0 | 0.2 | 2.0 | 20 |
| | **Fetal body weight** *(percent change compared to control)* | | | | |
| | | 0% | 2% | 3% | −7% |
| | Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d. | | | | |
| Angerhofer et al. (1986)<br>Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group)<br>Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants<br>0, 2, 6, or 20 mg/kg-d<br>Gavage<br>GDs 6–15 | Doses | 0 | 2 | 6 | 20 |
| | **Fetal body weight** *(percent change compared to control)* | | | | |
| | | 0% | −4% | −2% | −9%[a] |
| | **Fetal body length** *(percent change compared to control)* | | | | |
| | | 0% | −1% | −1% | −5%[b] |
| | Significant maternal mortality (16/51) occurred at 20 mg/kg-d. | | | | |
| *Morphological development* | | | | | |
| Cholakis et al. (1980)<br>Rabbits, New Zealand White, 11–12/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 7–29 | Doses | 0 | 0.2 | 2.0 | 20 |
| | **Spina bifida** *(incidence)* | | | | |
| | Fetuses | 0/88 | 0/99 | 0/94 | 3/110 |
| | Litters | 0/11 | 0/11 | 0/11 | 2/12 |
| | **Misshapen eye bulges** *(incidence)* | | | | |
| | Fetuses | 0/88 | 0/99 | 0/94 | 3/110 |
| | Litters | 0/11 | 0/11 | 0/11 | 1/12 |
| | **Cleft palate** *(incidence)* | | | | |
| | Fetuses | 0/39 | 1/46 | 2/44 | 2/52 |
| | Litters | 0/11 | 1/11 | 1/11 | 1/12 |
| | **Enlarged front fontanel** *(incidence)* | | | | |
| | Fetuses | 0/49 | 5/53 | 2/50 | 8/58 |
| | Litters | 0/11 | 2/11 | 2/11 | 2/12 |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| Cholakis et al. (1980)<br>Rats, F344, 24–25 females/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants.<br>0, 0.2, 2.0, or 20 mg/kg-d<br>Gavage<br>GDs 6–19 | No gross or soft-tissue anomalies were seen in any exposure group.<br>No treatment-related increase in the incidence of litters with skeletal anomalies was observed.<br>Significant maternal mortality (7/24 dams) occurred at 20 mg/kg-d. | | | | |
| Angerhofer et al. (1986)<br>Rats, Sprague-Dawley, 39–51 mated females/group (25–29 pregnant dams/group)<br>Purity 90%; 10% HMX and 0.3% acetic acid occurred as contaminants<br>0, 2, 6, or 20 mg/kg-d<br>Gavage<br>GDs 6–15 | No treatment-related increase in the incidence of anomalies was observed. | | | | |
| | Doses | 0 | 2 | 6 | 20 |
| | **Total malformations** *(percent of fetuses with malformations)* | | | | |
| | | 1% | 1% | 0% | 2% |
| | Significant maternal mortality (16/51) occurred at 20 mg/kg-d. | | | | |

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Statistically significant dose-related trend ($p < 0.05$) by linear trend test, performed for this assessment. Average fetal weights or lengths for each litter comprised the sample data for this test.




M - Maternal mortality observed at the highest dose
[1] Statistically signficant dose-related trend (p <= 0.05) by linear trend test, performed for this assessment.

**Figure 1-3. Exposure response array of reproductive and developmental effects following oral exposure.**

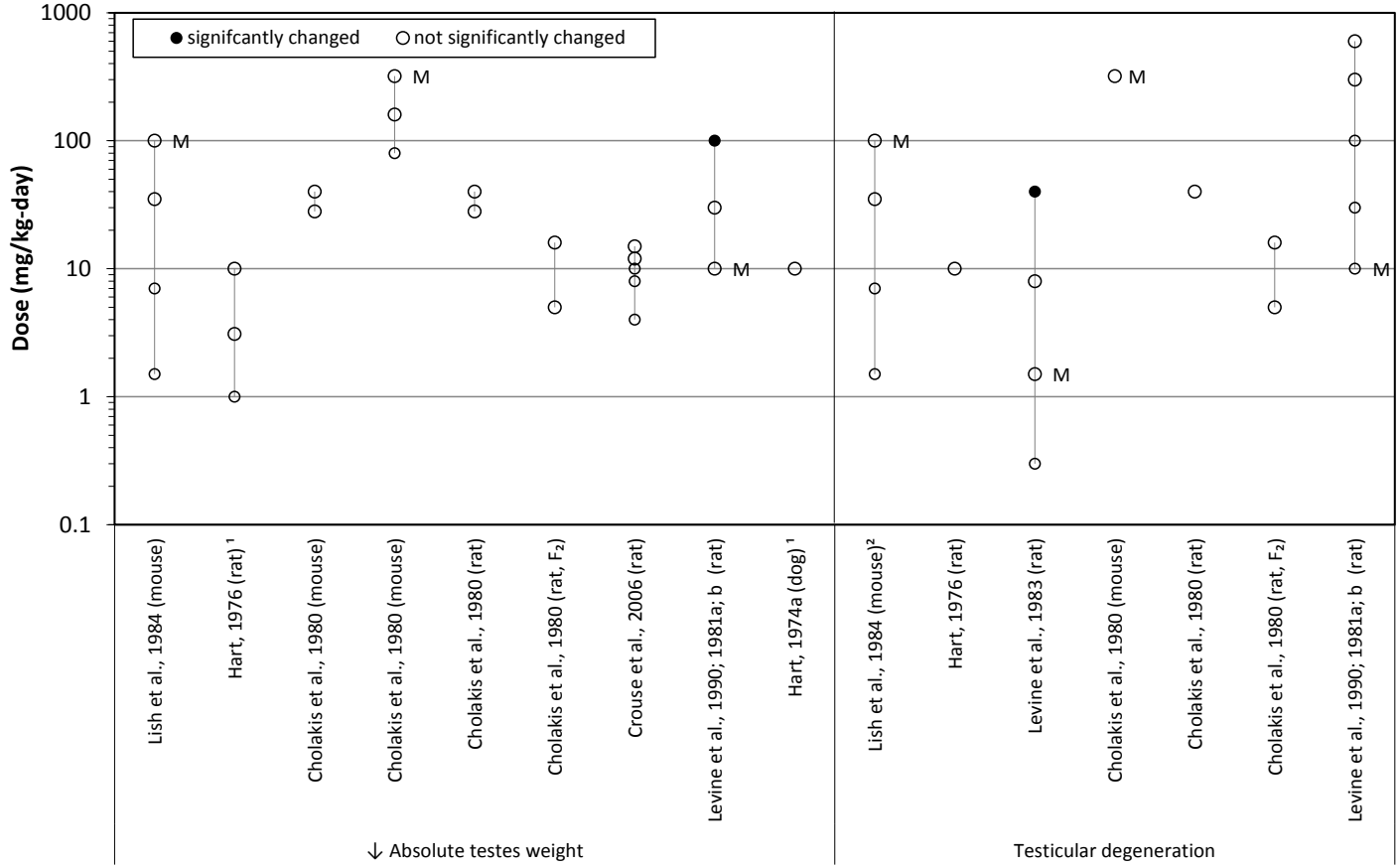
**Table 1-9. Evidence pertaining to male reproductive effects in animals**

| Reference and Study Design | Results | | | | | |
|---|---|---|---|---|---|---|
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Testicular degeneration** *(incidence)* | | | | | |
| | | 0/63 | 2/60 | 2/62 | 6/59 | 3/27[a] |
| | **Absolute testes weight; wk 105** *(percent change compared to control)* | | | | | |
| | | 0% | −6% | 0% | −2% | −6% |
| | **Relative testes weight; wk 105** *(percent change compared to control)* | | | | | |
| | | 0% | −4% | 2% | −2% | −2% |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/dose<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | 10 | |
| | **Absolute testes (with epididymis) weight; wk 104** | | | | | |
| | | 0% | −2% | 2% | 5% | |
| | **Relative testes (with epididymis) weight; wk 104** | | | | | |
| | | 0% | −1% | 7% | 9% | |
| | Testes were examined microscopically in control and 10 mg/kg-d groups; no degeneration or other treatment-related effects were observed. | | | | | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Testes, germ cell degeneration; 12 mo[b]** *(incidence)* | | | | | |
| | SS | 0/10 | 0/10 | 0/10 | 0/10 | 4/10* |
| | SDMS | – | – | 1/3 | – | 4/19 |
| | **Testes, germ cell degeneration; 24 mo** *(incidence)* | | | | | |
| | SS | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 |
| | SDMS | 0/16 | 0/19 | 0/27 | 0/26 | 0/27 |
| | Testes weights were not measured at termination due to testicular masses in nearly all males.<br>SDMS = spontaneous death or moribund sacrifice;<br>SS = scheduled sacrifice | | | | | |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>**Experiment 1**: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | – | – | – | −4% | −4% |
| | **Relative testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | – | – | – | 2% | −1% |
| **Experiment 2**: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or | Doses | 0 | 80 | 160 | 320 | | |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | | |

| Reference and Study Design | Results | | | | | |
|---|---|---|---|---|---|---|
| 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[d]<br>Diet<br>13 wks | | 0% | 4% | −4% | −8% | |
| | **Relative testes weight** *(percent change compared to control)* | | | | | |
| | | 0% | 1% | −4% | −9% | |
| | Testes were examined microscopically in control and 320 mg/kg day groups; no effects were observed. | | | | | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/dose<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | – | – | – | −2% | 0% |
| | **Relative testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | – | – | – | 2% | 9% |
| | Testes were examined microscopically in control and 40 mg/kg-d groups; no effects were observed. | | | | | | |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | In F2 offspring of 0, 5, and 16 mg/kg-d groups. No high-dose F2 animals available. | | | | | |
| | Doses | 0 | 5 | 16 | 50 | |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | |
| | | 0% | 3% | −31% | – | |
| | Testes were examined microscopically in all F2 groups; no effects observed. | | | | | |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | −3% | −5% | −4% | −4% | −8% |
| | **Relative testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | 4% | 5% | 0% | −6% | −10%* |
| Levine et al. (1981a); Levine et al. (1990); Levine et al. (1981b)[d]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Testes, germ cell degeneration** *(incidence)* | | | | | | |
| | | 0/10 | 0/10 | 0/10 | 0/10 | 1/9 | 1/10 |
| | **Absolute testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | 1% | 1% | −2% | – | – |
| | **Relative testes weight** *(percent change compared to control)* | | | | | | |
| | | 0% | 4% | 5% | 19%* | – | – |
| Hart (1974)<br>Dogs, Beagle, 3/sex/dose<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d | Doses | 0 | 0.1 | 1 | 10 | |
| | **Absolute testes (with epididymis) weight** *(percent change compared to control)* | | | | | |
| | | 0% | – | – | 51% | |

| Reference and Study Design | Results |
|---|---|
| Diet<br>90 d | Testes were not examined microscopically. |

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Although the study authors did not observe a statistically significant increase in the incidence of testicular degeneration, they determined that the incidences at the 35 and 175/100 mg/kg-day dose groups were "notable" when compared to concurrent (0%) and historical (1.5%) incidences.
[d]Testicular atrophy was observed at 12 months along with a statistically reduced mean testes weight (compared with controls). By 24 months, all male rats (including controls) had testicular masses; testes weights were not recorded, and an increased incidence of testicular degeneration was not observed.
[c]Doses were calculated by the study authors.
[d]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.




[1] Increased absolute weight of testes and epididymis

[2]Although the study authors did not observe a statistically significant increase in the incidence of testicular degeneration, they determined that the incidences at the 35 and 175/100 mg/kg-day dose groups were "notable" when compared to concurrent (0%) and historical (1.5%) incidences.

**Figure 1-4. Exposure response array of male reproductive effects following oral exposure.**

***Summary of Reproductive and Developmental Effects***

Developmental studies in rats (Angerhofer et al., 1986; Cholakis et al., 1980) and rabbits (Cholakis et al., 1980) suggest that developmental effects related to offspring survival, growth, and morphological development were likely associated with severe maternal toxicity. Developmental effects were observed only at doses that caused maternal mortality. As noted in EPA's *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), where adverse developmental effects are produced only at doses that cause minimal maternal toxicity, developmental effects should not be discounted as being secondary to maternal toxicity; however, at doses causing excessive toxicity, as is the case with RDX, information on developmental effects may be difficult to interpret and of limited value. Therefore, EPA concluded that the evidence does not support developmental effects as a potential human hazard of RDX exposure.

Testicular effects were reported in male $B6C3F_1$ mice chronically exposed to RDX in the diet for 24 months (Lish et al., 1984). No other studies of equivalent duration were performed in mice to determine the consistency of this effect. Germ cell degeneration was observed in F344 rats at 12 months, but not at 24 months in a 2-year study (Levine et al., 1984). Other testicular effects were inconsistent across rat studies. Based on the evidence reported by Lish et al. (1984), EPA identified suggestive evidence of male reproductive effects as a potential human hazard of RDX exposure.

### 1.1.4. Liver Effects

The association between RDX exposure and changes in serum liver enzymes was examined in a single occupational epidemiology study. Case reports involving accidental exposure to RDX provide information on the potential for acute exposure to RDX to affect the liver in humans. In addition, organ weight, histopathology, and serum chemistry findings from experimental animal studies involving subchronic and chronic exposure to ingested RDX provide data relevant to an examination of the association between RDX exposure and liver effects. A summary of the liver effects associated with RDX exposure is presented in Tables 1-10 and 1-11 and Figure 1-5.

Reports in humans provide limited evidence of liver toxicity associated with acute exposure to RDX. Elevated serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were reported in several case reports of individuals who ingested unknown amounts of RDX (Küçükardali et al., 2003; Woody et al., 1986; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968) (see Appendix C, Section C.3). Liver biopsies did not reveal any abnormal observations (Stone et al., 1969). In other case reports, no significant changes in serum levels of liver enzymes were observed (Testud et al., 1996b; Ketel and Hughes, 1972). In a cross-sectional epidemiologic study of workers from five U.S. Army munitions plants (69 exposed to RDX alone and 24 to RDX and HMX; mean average exposure concentration was 0.28 $mg/m^3$) (Hathaway and Buck, 1977), serum chemistry analysis (including the serum liver enzymes AST,

ALT, and alkaline phosphatase (ALP)) revealed no statistically significant differences between exposed and unexposed workers (Table 1-10).

In experimental animals, the most consistent noncancer liver effect associated with RDX exposure is elevated liver weight in studies of subchronic exposure (Table 1-11 and Figure 1-5). Dose-related increases in absolute and relative liver weight were observed in male and female B6C3F$_1$ mice given RDX in the diet for 90 days (Cholakis et al., 1980), and in female F344 rats in two separate 90-day dietary studies of RDX (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980). In another 90-day study, only absolute liver weights were increased in female F344 rats exposed to RDX by gavage (Crouse et al., 2006). The magnitude of liver weight increases in B6C3F$_1$ mice and female F344 rats across these studies ranged from 4–29% in the high-dose groups. Male F344 rats did not exhibit similar increases in liver weight in other subchronic studies (Crouse et al., 2006; Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980). In male and female monkeys exposed subchronically to RDX, absolute liver weights were increased (6–16% relative to control at 1 and 10 mg/kg-day) (Martin and Hart, 1974) and similarly in male, but not female beagle dogs (53% relative to control in male dogs at 10 mg/kg-day) (Hart, 1974). Chronic RDX exposures in B6C3F$_1$ mice and F344 or Sprague Dawley rats showed a less consistent pattern of liver weight increases. Interpretation of liver weight increases in 2-year studies is complicated by the incidence of adenomas and carcinomas in each dose group; the apparent increase in liver weights in male and female mice exposed to RDX in diet (Lish et al., 1984) was reduced when mice with liver adenomas or carcinomas were removed from the analysis. In a 2-year rat study, absolute liver weight showed no dose-related changes; however, relative liver weights were increased in high-dose (40 mg/kg-day) males and females (by 11 and 18% compared to controls, respectively) (Levine et al., 1983). The changes in relative liver weight likely reflected the depressed weight gain in the high-dose rats (2 - 30% in males and 10 - 15% in females). Based on an evaluation of the relationship between organ weight and body/brain weight to determine which endpoint (organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio) is likely to more accurately detect target organ toxicity, Bailey et al. (2004) concluded that relative liver weights (expressed as organ to body weight ratios) were better modeled for quantitative analysis than organ weight alone, or organ-to-brain weight ratios.

Nonneoplastic histopathological changes in the liver were not associated with RDX exposure in the majority of experimental animal studies (Crouse et al., 2006; Levine et al., 1990; Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Hart, 1974; Martin and Hart, 1974; Von Oettingen et al., 1949), including 2-year oral studies in mice at doses up to 100 mg/kg-day (Lish et al., 1984) and in rats at doses up to 40 mg/kg-day (Levine et al., 1983). The few findings of liver lesions were reported in studies with more limited histopathological analyses, and were not confirmed in the studies with more complete histopathologic examination and longer exposure durations (Levine et al., 1984; Lish et al., 1984; Levine et al., 1983; Thompson, 1983; Von Oettingen et al., 1949). For example, the incidence of liver portal inflammation was increased in

female but not male rats exposed to 40 mg/kg-day in the diet for 90 days (Cholakis et al., 1980). There was an increase in the incidence of mild liver microgranulomas in female mice only (Cholakis et al., 1980) and karyomegaly of hepatocytes in male mice only exposed to 320 mg/kg-day RDX in the diet for 90 days (Cholakis et al., 1980). In both the rat and mouse studies by Cholakis et al. (1980), groups sizes were relatively small (n = 10/sex/group) and histopathologic findings were reported for the control and high-dose groups only. It should be noted that exposure to HMX, the primary contaminant in several of the RDX studies, was associated with histopathological changes in the livers of male rats fed doses ≥450 mg/kg-day for 13 weeks. Similar findings were not observed in the RDX studies, where the doses of RDX employed in the studies would have resulted in HMX exposures of ≤60 mg/kg-day. The contribution of HMX exposure to the overall liver findings in the studies of RDX toxicity is therefore expected to be negligible.

Clinical chemistry parameters, including serum ALT, AST, and ALP, showed no treatment-related changes indicative of liver toxicity. Statistically significant changes in these parameters in some subchronic and chronic toxicity studies in rats and mice were relatively small (generally <50% of the control mean), were not dose-related in most instances, and showed no consistent pattern of change between sexes or across studies.

Some subchronic and chronic oral toxicity studies in rats and mice reported dose-related changes in serum cholesterol and triglyceride levels; however, these changes were not consistently observed in males and females within the same study, and patterns of changes were not consistent across studies. Specifically, serum triglyceride levels were elevated (up to 41%) in female B6C3F$_1$ mice exposed to RDX in the diet for 2 years, although increases were not dose-related (Lish et al., 1984); male mice in the same study did not show a similar increase in triglycerides. In contrast, serum triglycerides showed dose-related decreases in male and female F344 rats (50–62% at the high doses) in a subchronic oral (dietary) study (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b). In a chronic toxicity study by the same investigators (Levine et al., 1983), serum triglyceride levels were generally decreased in male and female rats (52 and 51%, respectively, at the highest dose of 40 mg/kg-day); however, triglyceride levels across the four dose groups in this study did not show a dose-related response.

Serum cholesterol levels showed a dose-related increase (38% at the high dose of 100 mg/kg-day) in female B6C3F$_1$ mice exposed to RDX in the diet for 2 years (Lish et al., 1984); however, changes in cholesterol in male mice in the same study were not dose related. Changes in serum cholesterol in male and female F344 rats exposed to RDX in the diet for 2 years at doses up to 40 mg/kg-day (Levine et al., 1983), in rats exposed to RDX by gavage for 90 days at doses up to 15 mg/kg-day (Crouse et al., 2006), and in monkeys exposed to RDX in the diet for 90 days (Martin and Hart, 1974) were relatively small (within 38% of control mean) and were not dose related.

**Table 1-10. Evidence pertaining to liver effects in humans**

| Reference and study design | Results | | | |
|---|---|---|---|---|
| Hathaway and Buck (1977) (United States) Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate). Analysis group: limited to whites; 69 exposed to RDX alone and 24 exposed to RDX and HMX; 338 not exposed to RDX, HMX, or TNT. **Exposure measures**: Exposure determination based on job title and industrial hygiene evaluation. Exposed subjects assigned to two groups: less than the limit of detection (LOD) or ≥ 0.01 mg/m$^3$ (mean 0.28 mg/m$^3$). **Effect measures**: Liver function tests. **Analysis**: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and $\chi^2$ tests for comparison of proportions). | **Liver function tests in men; mean (standard deviation not reported)** | | | |
| | Test | Referent (n = 237) | RDX exposed: Undetected (n = 22) | RDX exposed: >0.01 mg/m$^3$ (n = 45) |
| | LDH | 173 | 191 | 174 |
| | Alkaline phosphatase | 82 | 78 | 80 |
| | ALA (SGOT) | 22 | 25 | 21 |
| | AST (SGPT) | 21 | 26 | 18 |
| | Bilirubin | 0.5 | 0.4 | 0.4 |
| | No differences were statistically significant as reported by study authors. Similar results in women. | | | |
| | **Liver function tests in men: prevalence of abnormal values** | | | |
| | Test (abnormal range) | Referent | RDX exposed: Undetected | RDX exposed: >0.01 mg/m$^3$ |
| | LDH (>250) | 2/237 | 1/22 | 0/45 |
| | Alkaline phosphatase (>1.5) | 34/237 | 1/22 | 6/45 |
| | AST (SGOT) (>35) | 20/237 | 4/22 | 2/45 |
| | ALT (SGPT) (>35) | 15/237 | 2/22 | 0/45 |
| | Bilirubin (>1.0) | 5/237 | 1/22 | 1/45 |
| | No differences were statistically significant as reported by study authors. Similar results in women. | | | |

**Table 1-11. Evidence pertaining to liver effects in animals**

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| *Liver weight* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Absolute liver weight at 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 28%* | 11% | 12% | 35%* |
| | F | 0% | 7% | 7% | 15% | 18%* |
| | **Relative liver weight at 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 32%* | 12% | 14% | 46%* |
| | F | 0% | 6% | 8% | 18% | 45%* |
| | Note: Percent change in liver weights of male and female mice was reduced in all dose groups when mice with liver tumors were removed from the analysis. | | | | | |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | 10 | |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | |
| | M | 0% | −6% | −6% | −6% | |
| | F | 0% | 7% | −11% | 1% | |
| | **Relative liver weight** *(percent change compared to control)* | | | | | |
| | M | 0% | −5% | −2% | −3% | |
| | F | 0% | 17% | −2% | 13% | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Absolute liver weight at 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 3% | −7% | 1% | −8% |
| | F | 0% | 1% | −4% | 3% | 0% |
| | **Relative liver weight at 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 1% | 0% | 2% | 11% |
| | F | 0% | 1% | −2% | 6% | 18%* |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>**Experiment 1**: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −6% | −5% |
| | F | 0% | – | – | – | −4% | −1% |
| | **Relative liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −4% | −4% |
| | F | 0% | – | – | – | −6% | 1% |
| | Doses | 0 | | 80 | 160 | | 320 |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| **Experiment 2**: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a]<br>Diet<br>13 wks | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 2% | | 12% | | 26%* |
| | F | 0% | 4% | | 9% | | 29%* |
| | **Relative liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 0% | | 9% | | 25%* |
| | F | 0% | 4% | | 4% | | 22%* |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −2% | −5% |
| | F | 0% | – | – | – | 6% | 4% |
| | **Relative liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 2% | 3% |
| | F | 0% | – | – | – | 10% | 11% |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 5 | | 16 | | 50 |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 7% | | −16% | | – |
| | F | 0% | 0% | | −14% | | – |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −6% | −9% | 0% | 7% | 5% |
| | F | 0% | 1% | 7% | 18%* | 15% | 28%* |
| | **Relative liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 0% | −1% | 2% | 5% | 2% |
| | F | 0% | 1% | −2% | 2% | −3% | 2% |
| Levine et al. (1981a); Levine et al. (1990); Levine et al. (1981b)[b]<br>Rats, F344, 3–4 wks old; 10/sex/group; 30/sex/group for controls<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d | Data were not reported for rats in the 300 or 600 mg/kg-d dose groups because all of the rats died before the 13-wk necropsy. | | | | | | |
| | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 5% | −1% | −2% | – | – |
| | F | 0% | 2% | 4% | 16%* | – | – |

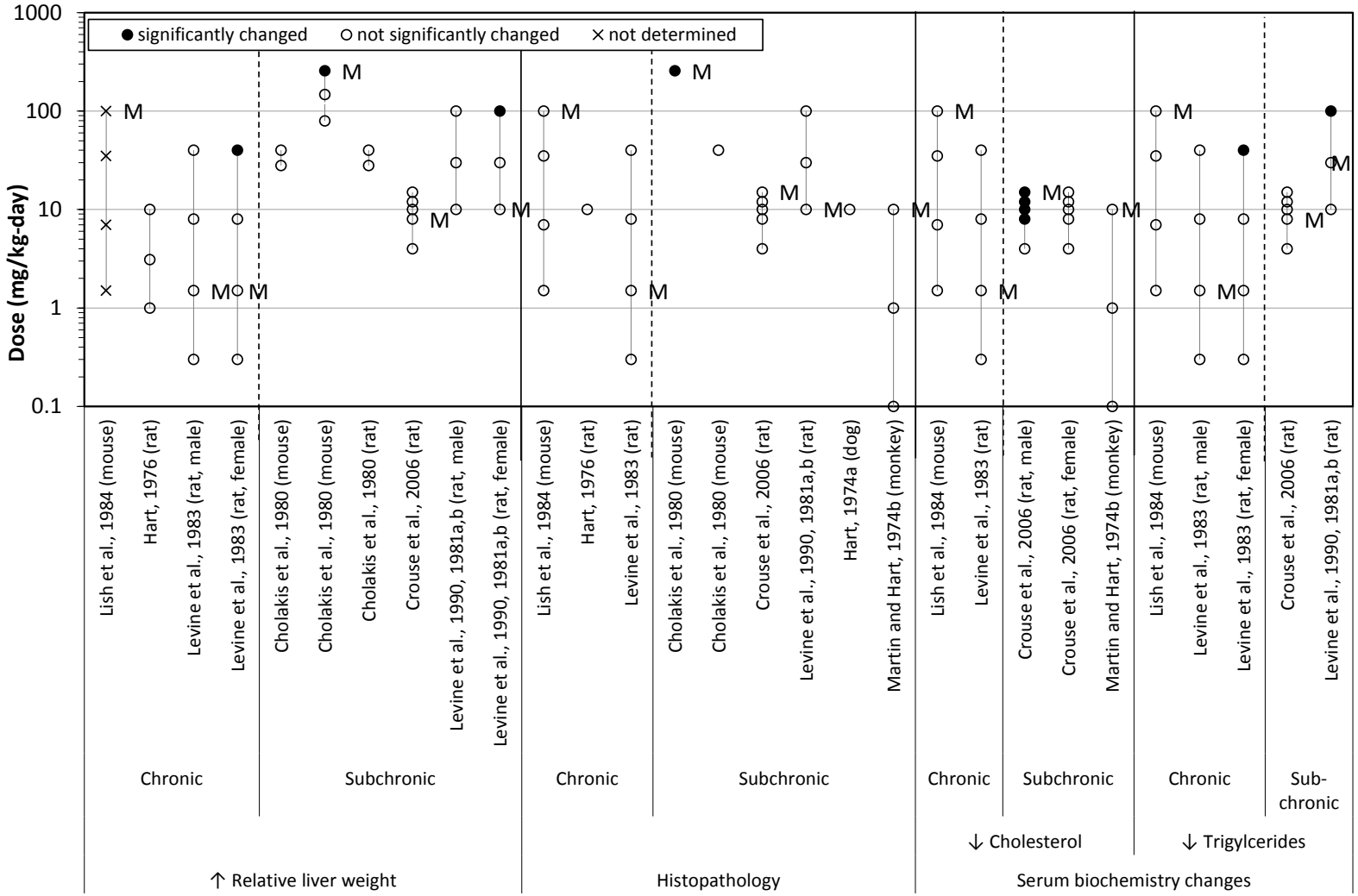
| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Diet<br>13 wks | **Relative liver weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 9% | 6% | 20% | – | – |
| | F | 0% | 3% | 5% | 19%* | – | – |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Doses | 0 | 0.1 | 1 | 10 | | |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | 53% | | |
| | F | 0% | – | – | 3% | | |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 0.1 | 1 | 10 | | |
| | **Absolute liver weight** *(percent change compared to control)* | | | | | | |
| | M + F | 0% | 2% | 6% | 16% | | |
| *Histopathological lesions* | | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Histopathological lesions in liver other than adenomas and carcinomas were not significantly different compared to controls, as reported by study authors. | | | | | | |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Histopathological examination performed only for controls and 10 mg/kg-d rats; no significant differences compared to controls were reported by study authors. | | | | | | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 3–4 wks old; 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 | |
| | **Microgranulomas** *(incidence)* | | | | | | |
| | M | 0/38 | 0/36 | 0/25 | 0/29 | 0/4 | |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| 89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | F | 10/43 | | 19/45 | 12/42 | 17/41 | 4/28 |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 80, 60, or 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[a]<br>Diet<br>13 wks | Doses | 0 | | 80 | 160 | | 320 |
| | **Liver microgranulomas; mild** *(incidence)* | | | | | | |
| | M | 2/10 | | – | – | | 1/9 |
| | F | 2/11 | | – | – | | 7/11* |
| | **Increased karyomegaly of hepatocytes** | | | | | | |
| | M | 0/10 | | – | – | | 5/9* |
| | F | – | | – | – | | – |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Liver granulomas; mild** *(incidence)* | | | | | | |
| | M | 0/10 | – | – | – | – | 1/10 |
| | F | – | – | – | – | – | – |
| | **Liver portal inflammation** | | | | | | |
| | M | 2/10 | – | – | – | – | 3/10 |
| | F | 1/10 | – | – | – | – | 7/10 |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Histopathology examination of the 15 mg/kg-d group showed one male rat with mild liver congestion and one female rat with a moderate-sized focus of basophilic cytoplasmic alteration; neither finding was attributed by study authors to RDX treatment. | | | | | | |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[b]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Histopathological examination of liver did not reveal any significant differences compared to controls, as reported by study authors. | | | | | | |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Histopathological examination performed only for controls and 10 mg/kg-d dogs; no significant differences compared to controls were reported. | | | | | |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | An increase in the amount of iron-positive material in liver cord cytoplasm was reported in monkeys treated with 10 mg/kg-d RDX; however, the study authors considered the toxicological significance to be uncertain. | | | | | |
| *Serum chemistry* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Serum cholesterol at 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 11% | −11% | 5% | 39% |
| | F | 0% | 5% | 15% | 25% | 38% |
| | **Serum triglycerides at 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 21% | −20% | 10% | −25% |
| | F | 0% | 34% | 28% | 41% | 28% |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Serum cholesterol at 104 wks (percent change compared to control)** | | | | | |
| | M | 0% | 15% | 38% | 19% | −6% |
| | F | 0% | 6% | 3% | −7% | −9% |
| | **Serum triglycerides at 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 14% | −15% | −12% | −52% |
| | F | 0% | 18% | 5% | −42% | −51%* |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Serum cholesterol** *(percent change compared to control)* | | | | | | |
| | M | 0% | −3% | −10%* | −16%* | −18%* | −11%* |
| | F | 0% | −1% | −8% | −4% | −4% | −1% |
| | **Serum triglycerides** *(percent change compared to control)* | | | | | | |
| | M | 0% | 1% | 1% | −7% | −2% | −19% |
| | F | 0% | −16% | −21% | 7% | −37% | 18% |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[b]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Data not reported for 300 and 600 mg/kg-d dose groups because all of the animals died before the 13-wk blood sampling. | | | | | |
| | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Serum triglyceride levels** *(percent change compared to control)* | | | | | | |
| | M | 0% | −14% | −34% | −62%* | – | – |
| | F | 0% | −12% | −29% | −50%* | – | – |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Serum biochemistry analysis revealed scattered deviations, but study authors indicated they appear to have no toxicological significance. | | | | | |
| | Doses | 0 | 0.1 | 1 | 10 | | |
| | **Serum cholesterol** *(percent change compared to control)* | | | | | | |
| | M | 0% | −17% | −2% | −7% | | |
| | F | 0% | 7% | 7% | 7% | | |

*Statistically significant ($p < 0.05$) based on analysis by study authors.
[a]Doses were calculated by the study authors.
[b]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.




X- not determined due to confounding caused by presence of tumors
These studies were excluded from array because only absolute liver weight was reported: Cholakis, 1980 (2-gen rat); Hart, 1974; Martin and Hart, 1974
M - Mortality observed at this dose and above

**Figure 1-5. Exposure response array of liver effects following oral exposure.**

***Summary of Liver Effects***

There is limited evidence from reports of human exposure and from studies in experimental animals that RDX may affect the liver. Several human case reports of short-term elevations of serum liver enzymes in individuals who ingested unknown amounts of RDX suggest that RDX might target the liver; however, serum liver enzymes were not elevated in a small prevalence study of munition plant workers exposed to RDX. In experimental animals, dose-related increases in relative or absolute liver weight were observed in multiple studies following subchronic oral exposure, in multiple species (mice, rats, dogs, and monkeys), and in both sexes; however, an association between RDX exposure and increased liver weight was not similarly supported by lifetime studies in mice and rats. Changes in serum liver enzymes were not consistent across studies and the magnitude of change relative to concurrent controls was not indicative of liver damage. Nonneoplastic histopathologic lesions of the liver were also not consistently associated with RDX exposure. EPA concluded that the evidence does not support liver effects as a potential human hazard of RDX exposure.

### 1.1.5. Carcinogenicity

The relationship between exposure to RDX and cancer in human populations has not been investigated. The carcinogenicity of RDX has been examined in one oral chronic/carcinogenicity bioassay in mice (Lish et al., 1984) and two bioassays in rats (Levine et al., 1983; Hart, 1976). The 2-year studies by Lish et al. (1984) and Levine et al. (1983) were performed in accordance with FDA Good Laboratory Practice regulations (FDA, 1979) and included comprehensive histopathological examination of major organs, multiple dose groups and a control, and more than 50 animals/dose group (plus additional interim sacrifice groups). The Hart (1976) study is largely limited by lack of characterization of the test material and pathology analysis limited to the control and high-dose groups. A temperature spike in the animal rooms on study day 76 resulted in significant mortality across all dose groups and control animals; however, there were still more than 80 rats/sex/group after the overheating incident and ≥50 rats/sex/group at termination, and it seems unlikely that the mortality associated with the temperature spike would have affected a tumor response in the rats. A summary of the evidence for liver and lung tumors in experimental animals from these three bioassays is provided in Tables 1-12 and 1-13.

Liver tumors

Increased incidence of liver tumors was observed in one chronic mouse study and one of two chronic rat studies. In the chronic mouse dietary study (Lish et al., 1984), the combined incidences of hepatocellular adenomas or carcinomas were increased with increasing RDX doses in female B6C3F$_1$ mice as compared to concurrent controls, but not in male B6C3F$_1$ mice similarly exposed to RDX for 2 years. In addition, the incidence of hepatocellular carcinomas showed a cant positive trend with dose in male, but not female, F344 rats exposed to RDX in the diet for 2 years (Levine et al., 1983) (Cochran-Armitage trend test performed for this review, p = 0.032). On the

other hand, there were no increased incidences of hepatocellular adenomas or carcinomas in Sprague-Dawley rats of either sex exposed to RDX via diet for two years at doses up to 10 mg/kg-day (Hart, 1976). Incidences of hepatocellular neoplasms are presented in Table 1-12. The tumor responses are discussed in further detail below.

In the female B6C3F$_1$ mouse study by Lish et al. (1984), the finding of a statistically significant increase in hepatocellular tumors may have been influenced by the incidence of hepatocellular adenomas/carcinomas in the concurrent female control mice, which the study authors noted was relatively low (1/65). However, as noted by the authors, the incidence of hepatocellular adenomas or carcinomas at RDX doses ≥35 mg/kg-day (19% at both doses) was also statistically significantly elevated when compared to the mean historical control incidence for female B6C3F$_1$ mice in National Toxicology Program (NTP) studies (147/1781 or 8%; range: 0–20%) (Haseman et al., 1985).[5]

A Pathology Working Group (PWG) review of the slides of female mouse liver lesions from the Lish et al. (1984) study resulted in some changes in lesion diagnosis (Parker et al., 2006; Parker, 2001). Some malignant tumors were downgraded to benign status and several lesions initially characterized as tumors were changed to non-tumors based on more recent diagnostic criteria used by the PWG (Harada et al., 1999). There was a statistically significant trend in the combined incidence of hepatocellular adenomas or carcinomas (using a Cochran-Armitage one-sided trend test performed by EPA), consistent with the original findings of Lish et al. (1984). Because the PWG analysis reflects more recent histopathological criteria for the grading of tumors, the incidence of hepatocellular adenomas or carcinomas as reported by Parker et al. (2006) were considered the more reliable measure of liver tumor response in female mice from the Lish et al. (1984) bioassay.

As noted above, male F344 rats showed a positive trend with dose in the incidence of hepatocellular carcinomas in the Levine et al. (1983) bioassay; however, the association with exposure is not strong, in part reflecting the lower magnitude of response. There were only a few tumors observed in the exposed groups (0/55, 0/52, 2/55, 2/31) relative to the control (1/55), as compared with the mice. There is less confidence that the final incidence in the highest-dose group accurately reflects lifetime cancer incidence because of low survival and no time-to-death information to estimate mortality-adjusted incidences; the available information may underestimate lifetime cancer incidence by overestimating the number of rats truly at risk. Some perspective on the magnitude of response is provided by comparing with incidence rates in

[5]Comparison of control incidences of hepatocellular adenomas or carcinomas between Lish et al. (1984) and Haseman et al. (1985) must be interpreted with caution because of cross-study differences in labs, diets, and sources of animals. Specifically, the labs used by NTP and analyzed by Haseman et al. (1985) did not include the lab contracted to perform the Lish et al. (1984) study, and it is not clear if the diet used in the Lish et al. (1984) study was included in the diets reported in the NTP studies. Further, the NTP studies included three different suppliers of mice; one supplier was also used in the Lish et al. (1984) study. EPA *Guidelines for Carcinogenic Risk Assessment* (U.S. EPA, 2005a) also note that, unless the tumor is rare, the standard for determining statistical significance of tumor incidence is a comparison of dosed animals with the concurrent controls.

historical controls, despite the limitation of this comparison due to the historical data originating from a different laboratory. In a paper published concurrently with the Levine et al. (1983) study, the NTP reported an incidence of liver carcinomas in untreated control male F344 rats of 0.7% (12/1,719; range: 0–2%) (Haseman et al., 1985). The incidence of liver carcinomas in control male rats in Levine et al. (1983) was at the upper end of the NTP range, and higher than the NTP range in the highest two dose groups. Nonmalignant liver tumors (neoplastic nodules) in F344 male rats in the historical controls were reported more frequently than carcinomas, with an average incidence of 3.5% (61/1,719; range: 0–12%) (Haseman et al., 1985); Levine et al. (1983) reported a higher incidence of neoplastic nodules, 7.3%, in their control male rats, with a decline in incidence with increasing RDX exposure. Although there are several reasons to conclude that the observation of an association between RDX exposure and liver tumors in rats is not strong, this suggestive site concordance supports the response in female mice.

**Table 1-12. Liver tumors observed in chronic animal bioassays**

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Hepatocellular adenomas** *(incidence)*[a] | | | | | |
| | M | 8/63 | 6/60 | 1/62* | 7/59 | 7/27 |
| | F | 1/65 | 1/62 | 6/64 | 6/64 | 3/31[b] |
| | **Hepatocellular carcinomas** *(incidence)*[a] | | | | | |
| | M | 13/63 | 20/60 | 16/62 | 18/59 | 6/27 |
| | F | 0/65 | 4/62 | 3/64 | 6/64 | 3/31[a] |
| | **Hepatocellular adenoma or carcinoma combined** *(incidence)*[a] | | | | | |
| | M | 21/63 | 26/60 | 17/62 | 25/59 | 13/27 |
| | F | 1/65 | 5/62 | 9/64* | 12/64* | 6/31*[b] |
| | Pathology workgroup reanalysis of liver lesion slides from female mice (Parker et al., 2006; Parker, 2001)[c] | | | | | |
| | Doses | 0 | 1.5 | 7.0 | 35 | 175 |
| | **Hepatocellular adenomas** *(incidence)*[a] | | | | | |
| | F | 1/67 | 3/62 | 2/63 | 8/64 | 2/31[b] |
| | **Hepatocellular carcinomas** *(incidence)*[a] | | | | | |
| | F | 0/67 | 1/62 | 3/63 | 2/64 | 2/31[b] |
| | **Hepatocellular adenoma or carcinoma combined** *(incidence)*[a] | | | | | |
| | F | 1/67[c] | 4/62 | 5/63[c] | 10/64 | 4/31[b] |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group | **Doses** | 0 | 1.0 | 3.1 | | 10 |
| | **Neoplastic nodules** *(incidence)*[a] | | | | | |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | M | 0/82 | – | – | 3/77 | |
| | F | 1/72 | – | – | 1/81 | |
| | **Hepatocellular carcinomas** *(incidence)*[a] | | | | | |
| | M | 1/82 | – | – | 1/77 | |
| | F | 1/72 | – | – | 1/81 | |
| | **Neoplastic nodules or hepatocellular carcinomas combined** *(incidence)*[a] | | | | | |
| | M | 1/82 | – | – | 4/77 | |
| | F | 2/72 | – | – | 2/81 | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Neoplastic nodules** *(incidence)*[a] | | | | | |
| | M | 4/55 | 3/55 | 0/52 | 2/55 | 1/31 |
| | F | 3/53 | 1/55 | 1/54 | 0/55 | 4/48 |
| | **Hepatocellular carcinomas** *(incidence)*[a] | | | | | |
| | M | 1/55 | 0/55 | 0/52 | 2/55 | 2/31[b] |
| | F | 0/53 | 1/55 | 0/54 | 0/55 | 0/48 |
| | **Neoplastic nodules or hepatocellular carcinomas combined** *(incidence)*[a] | | | | | |
| | M | 5/55 | 3/55 | 0/52 | 4/55 | 3/31 |
| | F | 3/53 | 2/55 | 1/54 | 0/55 | 4/48 |

*Statistically significant difference compared to the control group ($p < 0.05$), identified by the authors.
[a]The incidences reflect the animals surviving to month 12.
[b]Statistically significant trend ($p < 0.05$) was identified using Cochran-Armitage trend tests performed by EPA.
[c]It is not clear why the numbers of animals at risk in the control group (n = 67) and 7 mg/kg-day dose group (n = 63) differed from the numbers reported in the original study (n = 65 and 64, respectively).

Lung tumors

Cochran-Armitage trend tests (as performed for this review, p = 0.019) found statistically significant positive trends in the incidences of alveolar/bronchiolar adenomas in female B6C3F$_1$ mice, alveolar/bronchiolar carcinomas in male mice, and alveolar/bronchiolar adenomas or carcinomas combined in female mice. The combined incidence in male B6C3F$_1$ mice did not show a statistically significant trend (see Table 1-13). In an addendum to the study report that included results of additional examination and sectioning of lung specimens from the mid-dose groups in the mouse study, Lish et al. (1984) noted an increase in the combined incidences of primary pulmonary neoplasms in males of all dose groups and in females in the 7.0, 35, and 175/100 mg/kg-day dose groups. However, the authors regarded these neoplasms as random and not biologically significant.

Bioassays in rats provide no evidence of an association between RDX exposure and induction of lung tumors. The incidence of alveolar/bronchiolar adenomas or carcinomas was not increased in either sex of Sprague-Dawley rats exposed chronically to RDX at doses up to 10 mg/kg-day (Hart, 1976) or in F344 rats of either sex exposed chronically to RDX at doses up to 40 mg/kg-day (Levine et al., 1983).

**Table 1-13. Lung tumors observed in chronic animal bioassays**

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Alveolar/bronchiolar adenomas** *(incidence)*[a] | | | | | |
| | M | 6/63 | 5/60 | 5/62 | 7/59 | 1/27 |
| | F | 4/65 | 2/62 | 5/64 | 9/64 | 3/31[b] |
| | **Alveolar/bronchiolar carcinomas** *(incidence)*[a] | | | | | |
| | M | 3/63 | 6/60 | 3/62 | 7/59 | 5/27[b] |
| | F | 3/65 | 1/62 | 3/64 | 3/64 | 4/31 |
| | **Alveolar/bronchiolar adenoma or carcinoma combined** *(incidence)*[a] | | | | | |
| | M | 9/63 | 11/60 | 8/62 | 14/59 | 6/27 |
| | F | 7/65 | 3/62 | 8/64 | 12/64 | 7/31[b] |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | | 10 |
| | **Alveolar/bronchiolar adenoma** *(incidence)* | | | | | |
| | M | 2/83 | – | – | | 1/77 |
| | F | 0/73 | – | – | | 0/82 |
| | **Alveolar/bronchiolar carcinoma** *(incidence)*<br>None reported by study authors. | | | | | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Alveolar/bronchiolar adenomas** *(incidence)*[a] | | | | | |
| | M | 1/55 | 0/15 | 1/17 | 0/16 | 1/31 |
| | F | 3/53 | 0/7 | 0/8 | 1/10 | 0/48 |
| | **Alveolar/bronchiolar carcinomas** *(incidence)*[a] | | | | | |
| | M | – | – | – | – | – |
| | F | 0/53 | 0/7 | 1/8 | 0/10 | 0/48 |
| | **Alveolar/bronchiolar adenoma or carcinoma combined** *(incidence)*[a] | | | | | |
| | M | – | – | – | – | – |
| | F | 3/53 | 0/7 | 1/8 | 1/10 | 0/48 |

[a]The incidences reflect the animals surviving to month 12.
[b]Statistically significant trend ($p < 0.05$) was identified using Cochran-Armitage trend test performed by EPA.

***Mechanistic Evidence***

There are few mechanistic data to support a MOA determination for either liver or lung tumors induced by exposure to RDX.

The increase in liver weights observed in subchronic studies of RDX in mice (Cholakis et al., 1980) and rats (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980) and chronic studies in female B6C3F$_1$ mice (Lish et al., 1984; Cholakis et al., 1980) raises the possibility of RDX-related liver cell proliferation as a precursor to tumorgenicity. Sweeney et al. (2012b) reviewed hypothesized MOA's for carcinogenicity and concluded that a MOA involving a proliferative response generated by tissue-derived oxidative metabolites of RDX was the most plausible of the MOAs considered, but acknowledged that the overall support for this MOA was limited. The following lines of evidence do not support a metabolite-based proliferative response as the MOA for RDX carcinogenicity:

- the absence of significant liver histopathology in mice after subchronic or chronic exposure to RDX at doses that induced liver tumors (Lish et al., 1984; Cholakis et al., 1980) suggests that cellular toxicity is not a precursor to these tumors;
- increased liver weight was also observed in rats and male mice where tumors did not occur;
- no studies were available that directly measured RDX-induced cell proliferation rates; and
- no information was available to rule out non-precancerous causes of liver weight increase.

The available in vitro and in vivo genotoxicity assay results are largely negative for parent RDX (see Appendix C, Section C.4), supporting the hypothesis that parent RDX does not interact directly with DNA. Sweeney et al. (2012b) proposed that the increased incidence of liver adenomas and carcinomas in female mice (Parker et al., 2006; Lish et al., 1984) may result from liver-generated metabolites as the most likely agents responsible for liver tumors. Sweeney et al. (2012b) estimated an approximately 30-fold higher metabolic rate for RDX in mice (which displayed a more robust liver tumor response to RDX exposure than did rats) compared with rats based on the results of a PBPK model. These authors hypothesized a non-linear, cell proliferation MOA in conjunction with the lack of evidence to support a genotoxic/mutagenic MOA for RDX or its oxidative metabolites. Sweeney et al. (2012b) suggest that RDX is unlikely to be genotoxic because it does not induce tumors at multiple sites and species. This observation is inconsistent with the finding in Lish et al. (1984) that showed positive trends in the incidence of both alveolar/bronchiolar adenomas or carcinomas and liver tumors.

In contrast to the negative results for RDX oxidative metabolites, there are some positive genotoxicity results for the *N*-nitroso metabolites of RDX, specifically hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine [MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). MNX and TNX have been identified from minipigs; minipigs were chosen as the animal model for the metabolism of RDX because the gastrointestinal tract of pigs more closely resembles that of humans

(Musick et al., 2010; Major et al., 2007). MNX has tested positive in some in vitro assays, including unscheduled DNA synthesis in primary rat hepatocytes and the mouse lymphoma forward mutation assay (Snodgrass, 1984), although MNX tested negative in the only in vivo test performed, a mouse dominant lethal mutation test (Snodgrass, 1984). MNX was not mutagenic in *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), with or without the addition of the S9 metabolic activating mixture (Pan et al., 2007; Snodgrass, 1984). When *S. typhimurium* strains TA97a and TA102, strains sensitive to frame shift and oxidative DNA damage, were used in conjunction with elevated concentrations of the metabolizing system (S9), MNX and TNX were mutagenic. *N*-nitroso metabolites, including MNX and TNX, are generated anaerobically and are likely a result of bacterial transformation of parent RDX in the gastrointestinal tract to various *N*-nitroso derivatives (Pan et al., 2007). Exposure to potentially mutagenic N-nitroso metabolites of RDX generated in the gastrointestinal tract of mice may occur in the liver (and subsequently in the systemic circulation) via enterohepatic circulation. However, in pigs the N-nitroso metabolites of RDX have been identified only in trace amounts in urine compared to the major metabolites, 4-nitro-2,4-diazbutanal and 4-nitro-2,4-diazbutanamide. Thus, the contribution of the N-nitroso metabolites to the overall carcinogenic potential of RDX is unclear.

Aberrant expression of microRNAs (miRNAs) was observed in the brains and livers of female B6C3$F_1$ mice fed 5 mg RDX/kg in the diet for 28 days (Zhang and Pan, 2009b), with several oncogenic miRNAs being upregulated, while several tumor-suppressing miRNAs were down regulated. However, the pattern of induction was not always consistent in the livers of RDX-treated mice (e.g., mIR-92a was downregulated in liver tissue samples when it is typically upregulated in hepatocellular carcinomas) (Sweeney et al., 2012b). miRNAs have been associated with several cancers (Wiemer, 2007; Zhang et al., 2007); however, the utility of miRNAs as predictive of carcinogenesis has not been established, and whether or not aberrant expression of a specific miRNA (or suite of miRNA's) plays a role in the MOA of RDX carcinogenicity is unknown. Microarray analysis of gene expression in male Sprague-Dawley rats after exposure to a single oral (capsule) dose of RDX revealed a general up-regulation in gene expression (predominantly genes involved in metabolism) in liver tissues (Bannon et al., 2009); however, the relevance of this finding to the carcinogenicity of RDX is unclear.

In summary, the available evidence indicates that RDX is not mutagenic (see Appendix C, Section C.4); however, anaerobically-derived N-nitroso metabolites have demonstrated some genotoxic potential. While these metabolites have been measured in the mouse (Pan et al., 2007) and minipig (Musick et al., 2010; Major et al., 2007), they have not been identified in humans, and may not be the predominant metabolites of RDX. A MOA involving a proliferative response generated by tissue-derived oxidative metabolites of RDX has been proposed, but is not supported by the available data. In light of limited information on precursor events leading to the observed liver and lung tumor response in RDX-exposed rodents and lack of toxicokinetic information on RDX metabolites, neither a cell proliferative MOA or a mutagenic N-nitroso metabolite MOA is

supported. Thus, the MOA leading to the increased incidence of liver and lungs tumors is not known.

### 1.1.6. Other Toxicological Effects

There is limited evidence that RDX can produce systemic effects in several organs/systems, including the eyes, and the musculoskeletal, cardiovascular, immune, and gastrointestinal systems. However, there is less evidence for these effects compared to organ systems described earlier in Section 1.1. A summary of the evidence for toxicological effects in other organ systems is shown in Tables 1-14 and 1-15.

#### *Ocular Effects*

There are no reports of ocular effects in human case reports or epidemiological studies. The incidence of cataracts was statistically significantly increased in high-dose female rats in one chronic oral study; however, this finding was not reproduced in other subchronic and chronic studies in rats or mice.

The incidence of cataracts was 73% in female F344 rats exposed to 40 mg/kg-day RDX in the diet for 2 years, compared to 32% in the control group (Levine et al., 1983). After 76 weeks of exposure, the incidence of cataracts in female rats at 40 mg/kg-day (23%) was also elevated compared to controls (6%). The incidence of cataracts was not increased in RDX-exposed male rats in the same study (Levine et al., 1983), and other studies have not observed ocular effects associated with RDX exposure. Only 2 rats (dose groups not reported) were observed to have mild cataracts in a 90-day study of male and female F344 rats exposed to RDX at doses up to 15 mg/kg-day by gavage; however, the authors noted that these observations are common in F344 rats at 4 months of age and should not be attributed to treatment (Crouse et al., 2006). Furthermore, cataracts were not observed in male or female F344 rats exposed to 40 mg/kg-day RDX by diet for 90 days (Cholakis et al., 1980), or in male or female B6C3$F_1$ mice exposed to RDX in the diet for 2 years at doses up to approximately 100 mg/kg-day (Lish et al., 1984). A statistically significant increase in the incidence of cataracts in male mice was initially noted by Lish et al. (1984), but was not confirmed when mice used for orbital bleedings were excluded from the analysis, suggesting the effect was not treatment related.

#### *Cardiovascular Effects*

Human evidence for cardiovascular effects is limited to case reports that include observations of transient arterial hypertension in male Italian workers following inhalation of RDX during manufacturing (Barsotti and Crotti, 1949), sinus tachycardia, and in one instance premature ventricular beats in 5 men following accidental ingestion of RDX at 37–250 mg/kg body weight (Küçükardali et al., 2003) (see Appendix C, Section C.3).

Inconsistent observations of cardiovascular effects have been reported in animal studies. An increase in the relative heart-to-body weight ratio was observed at the highest dose tested in

B6C3F$_1$ mice (male: 13%; female 17%) and F344 rats (male: 22%; female 15%) following chronic dietary administration of RDX (Lish et al., 1984; Levine et al., 1983); however, this dose also resulted in reductions in body weight in both males and females. Dose-related decreases in absolute heart weight were reported following subchronic exposures to RDX in the diet (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980), while a subchronic study in male dogs reported a 31% increase in absolute heart weight at the highest dose tested (10 mg/kg-day) (Hart, 1974).

Evidence for histopathologic changes associated with RDX exposure is limited to findings of an increased incidence of focal myocardial degeneration in female rats compared to controls (60 vs. 20%, respectively) and male mice (50 vs. 0%, respectively) following exposure to RDX in the diet for 90 days (Cholakis et al., 1980). In each study, the finding of myocardial degeneration was limited to one sex and to the high-dose group only. Other studies in monkeys (Martin and Hart, 1974) and rats (Von Oettingen et al., 1949) reported no observable cardiovascular effects.

***Musculoskeletal Effects***

Evidence of musculoskeletal effects in humans consists of case reports that include observations of muscle twitching, myalgia/muscle soreness, and muscle injury as indicated by elevated levels of AST or myoglobinuria (Küçükardali et al., 2003; Hett and Fichtner, 2002; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968) (see Appendix C, Section C.3).

Histological evaluations of musculature or skeletal tissue did not reveal any alterations in mice (Lish et al., 1984) or rats (Levine et al., 1983; Hart, 1976) following chronic oral exposure to RDX, in mice and rats following subchronic exposure (Cholakis et al., 1980), or in dogs following a 90-day dietary exposure (Hart, 1974).

***Immune Effects***

RDX is structurally similar to various drugs known to induce the autoimmune disorder systemic lupus erythematosus (SLE). Three cases of SLE were initially identified among workers at one U.S. Army munitions plant; however, upon further investigation of 69 employees at five U.S. Army munitions plants with potential exposure to RDX, no additional cases of SLE were identified (Hathaway and Buck, 1977). Increased white blood cell (WBC) counts have been reported in some case reports of individuals who ingested RDX or C-4 (91% RDX) (Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968).

In animal studies, increased WBC count in female rats following subchronic dietary exposure to RDX was the only dose-related immune effect reported (Levine et al., 1990; Levine et al., 1981a; Levine et al., 1981b); WBC counts in male rats were unaffected. Conversely, decreased WBC counts in were reported in male and female rats in a 2-year study (Hart, 1976). Changes in spleen weights were observed across studies, but the responses were not consistent and did not appear to be dose-related. For example, in 90-day studies, Cholakis et al. (1980) identified a statistically significant decrease in absolute spleen weight in F344 rats at 40 mg/kg-day, while

Crouse et al. (2006) observed an increase in spleen weight at 15 mg/kg-day (not statistically significant). Across studies, there was no significant or dose-dependent pattern of response to suggest that the WBC changes reflect RDX-induced immunotoxicity. No dose-related immune effects from oral exposure to RDX were observed in other animal studies, including a 90-day study in F344 rats specifically designed to evaluate immunotoxicity (parameters included evaluation of red and white blood cell populations, proportion of cell surface markers, cellularity in proportion to organ weight, B and T cells in the spleen, and CD4/CD8 antigens of maturing lymphocytes in the thymus) (Crouse et al., 2006). Routine clinical and histopathology evaluations of immune-related organs in a two-generation study in rats (Cholakis et al., 1980) and chronic studies in rats (Levine et al., 1983) and mice (Lish et al., 1984) provide no evidence of immunotoxicity associated with oral (dietary) exposure to RDX.

In summary, evidence for immunotoxicity associated with RDX exposure is limited to findings from one study of increased WBC counts in female rats (Levine et al., 1981a; Levine et al., 1981b). Evidence that RDX is not immunotoxic comes from several animal studies, including other repeat-dose oral studies in mice and rats (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Cholakis et al., 1980).

### *Gastrointestinal Effects*

Clinical signs of nausea and/or vomiting have been frequently identified in case reports of accidental or intentional RDX poisonings, and generally concurrent with severe neurotoxicity (Kasuske et al., 2009; Davies et al., 2007; Küçükardali et al., 2003; Hett and Fichtner, 2002; Ketel and Hughes, 1972; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968; Kaplan et al., 1965; Barsotti and Crotti, 1949) (see Appendix C, Section C.3). In animal studies, nausea and vomiting have also been observed following oral exposure of swine (Musick et al., 2010), dogs (Hart, 1974), and monkeys (Martin and Hart, 1974). One subchronic oral (diet) rat study from the early literature reported congestion of the gastrointestinal tract at doses also associated with elevated mortality (Von Oettingen et al., 1949); however, none of the subsequent subchronic or chronic animal studies reported histological findings of the gastrointestinal tract related to RDX administered via gavage or the diet (Crouse et al., 2006; Lish et al., 1984; Levine et al., 1983; Hart, 1974; Martin and Hart, 1974).

### *Hematological Effects*

Elevated prevalence odds ratios (OR) for hematological abnormalities were observed in a case-control study of males (32 exposed, 322 controls) exposed to RDX in an occupational setting (West and Stafford, 1997) (see Table 1-14). The prevalence OR for an association between RDX exposure and hematological abnormalities was 1.7 (95% CI 0.7–4.2) for men with greater than 50 hours of low intensity exposure, while the prevalence OR was 1.2 (95% CI 0.3–5.3) for men with >50 hours of high intensity exposure. The ORs from this study must be interpreted with caution given the small sample size and wide confidence intervals. No changes in hematological parameters

(including hemoglobin, hematocrit, and reticulocyte count) were observed in a cross-sectional epidemiologic study of 69 workers exposed to RDX by inhalation (average of 0.28 mg/m$^3$) (Hathaway and Buck, 1977). Humans who ingested or inhaled unknown amounts of RDX or C-4 (~91% RDX) for an acute duration displayed temporary hematological alterations, including anemia, decreased hematocrit, hematuria, and methemoglobinemia (Kasuske et al., 2009; Küçükardali et al., 2003; Knepshield and Stone, 1972; Hollander and Colbach, 1969; Stone et al., 1969; Merrill, 1968). In other case reports, normal blood counts were observed in accidentally exposed individuals (Testud et al., 1996b; Goldberg et al., 1992; Woody et al., 1986; Ketel and Hughes, 1972; Kaplan et al., 1965) (see Appendix C, Section C.3).

In animals, hematological alterations were observed following oral exposure in chronic and subchronic studies in both sexes of rats (F344 or SD) and B6C3F$_1$ mice (see Table 1-15). Increases in platelet count were observed in male and female mice and rats in some subchronic and chronic studies at doses from 0.3 mg/kg-day to 320 mg/kg-day (Lish et al., 1984; Levine et al., 1983; Cholakis et al., 1980); however, findings were generally inconsistent across studies and were not necessarily dose-dependent. Similarly, decreased hemoglobin levels/anemia were observed in some chronic and subchronic studies (Levine et al., 1983; Cholakis et al., 1980; Von Oettingen et al., 1949), particularly at doses greater than or equal to 15 mg/kg-day, but trends in hemoglobin levels across studies did not show a consistent relationship with dose. Other hematological parameters, including WBC counts, reticulocyte counts, and hematocrit, showed conflicting results between studies, marginal responses, or inconsistent changes with increasing dose. Other subchronic studies in rats and dogs (Crouse et al., 2006; Hart, 1974; Von Oettingen et al., 1949) did not identify any changes in hematological parameters.

In summary, evidence for hematological effects associated with RDX exposure in humans comes from several case reports that found transient fluctuations in hematological endpoints after acute exposures. Hematological findings from two epidemiological studies were inconsistent and difficult to interpret because of small sample sizes (Table 1-14). In general, animal studies of chronic and subchronic durations showed no consistent, dose-related pattern of increase or decrease in hematological parameters.

**Table 1-14. Evidence pertaining to systemic effects (hematological) in humans**

| Reference and study design | Results | |
|---|---|---|
| *Hematological effects* | | |
| West and Stafford (1997) (United Kingdom) Case-control study, 32 cases with abnormal and 322 controls with normal hematology test drawn from 1991 study of 404 workers at ammunitions plant; participation rate 97% of | **Odds ratio (95% CI) [number of exposed cases] of blood disorder and RDX** | |
| | Low intensity, 50 hr-duration | 1.7 (0.7,4.2) [22] |
| | Medium intensity, 50-hr duration | 1.6 (not reported) [5] |

<table>
<tr><th>Reference and study design</th><th>Results</th></tr>
<tr><td colspan="2">Hematological effects</td></tr>
<tr><td>cases, 93% of controls. Analysis limited to men (29 cases, 282 controls).<br>Exposure measures: Exposure determination based on employee interviews and job title analysis; data included frequency (hrs/d, d/yr), duration (yrs), and intensity (low [1−10 ppm], moderate [10−100 ppm], and high [100−1,000 ppm], based on ventilation considerations).<br>Effect measures: Hematology tests; blood disorder defined as neutropenia (2.0 × $10^9$/L), low platelet count (<150 × $10^9$/L), or macrocytosis (mean corpuscular volume = 99 fl or >6% macrocytes).<br>Analysis: Unadjusted odds ratio.</td><td>High intensity, 50-hr duration 1.2 (0.3, 5.3) [2]</td></tr>
<tr><td>Hathaway and Buck (1977) (United States) Cross-sectional study, 2,022 workers, 1,491 participated (74% response rate). Analysis limited to whites; 69 exposed to RDX alone and 24 exposed to RDX and HMX; 338 not exposed to RDX, HMX, or TNT.<br>Exposure measures: Exposure determination based on job title and industrial hygiene evaluation. Exposed subjects assigned to two groups: <LOD or ≥ 0.01 mg/$m^3$ (mean 0.28 mg/$m^3$).<br>Effect measures: Hematology tests.<br>Analysis: Types of statistical tests were not reported (assumed to be t-tests for comparison of means and $\chi^2$ tests for comparison of proportions).</td><td>Hematology tests in men; mean (standard deviation not reported)
<table>
<tr><th rowspan="2">Test</th><th colspan="3">RDX exposed</th></tr>
<tr><th>Referent (n = 237)</th><th>Undetected (n = 22)</th><th>>0.01 mg/$m^3$ (n = 45)</th></tr>
<tr><td>Hemoglobin</td><td>15.2</td><td>14.7</td><td>15.2</td></tr>
<tr><td>Hematocrit</td><td>42</td><td>45.6</td><td>47</td></tr>
<tr><td>Reticulocyte count</td><td>0.7</td><td>0.9</td><td>0.7</td></tr>
</table>
No differences were statistically significant. Similar results in women.<br>Hematology tests in men: prevalence of abnormal values
<table>
<tr><th rowspan="2">Test (abnormal range)</th><th colspan="3">RDX exposed</th></tr>
<tr><th>Referent</th><th>Undetected</th><th>>0.01 mg/$m^3$</th></tr>
<tr><td>Hemoglobin (<14)</td><td>15/237</td><td>3/22</td><td>4/45</td></tr>
<tr><td>Hematocrit (<40)</td><td>1/237</td><td>1/22</td><td>1/45</td></tr>
<tr><td>Reticulocyte count (>1.5)</td><td>18/237</td><td>3/22</td><td>2/45</td></tr>
</table>
No differences were statistically significant. Similar results in women.</td></tr>
</table>

**Table 1-15. Evidence pertaining to systemic effects in animals**

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| *Ocular effects* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **Cataracts; 103 wks** *(incidence)*[a] | | | | | |
| | M | 2/47 | 2/41 | 0/41 | 2/37 | 2/16 |
| | F | 2/50 | 1/37 | 6/52 | 0/46 | 1/26 |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Cataracts; 103 wks** *(incidence)* | | | | | |
| | M | 8/40 | 6/39 | 6/31 | 8/35 | 2/6 |
| | F | 14/44 | 4/48 | 11/44 | 8/43 | 22/30* |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | No ocular effects were observed (gross examination of eye was performed in all animals, and microscopic examination in control and 40 mg/kg-d animals). | | | | | |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | No ocular effects were observed (ophthalmic examinations were performed in all animals within 1 wk of sacrifice, and microscopic examination of the eye was performed in control and 15 mg/kg-danimals). | | | | | |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | No ocular effects were observed (ophthalmoscopic examination was performed at the end of exposure). | | | | | |
| *Cardiovascular effects* | | | | | | |
| Lish et al. (1984); Levine et al. (1984) | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | **Absolute heart weight; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 4% | 4% | 5% | 7% |
| | F | 0% | 1% | 5% | 2% | −5% |
| | **Relative heart-to-body weight; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 7% | 5% | 5% | 13%* |
| | F | 0% | 0% | 6% | 4% | 17%* |
| | Body weight was significantly lower at termination in males and females exposed to 175/100 mg/kg-d (−5 and −19%, respectively). | | | | | |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | 10 | |
| | **Myocardial fibrosis** *(percent incidence; number not reported)* | | | | | |
| | M | 20% | – | – | 5% | |
| | F | 5% | – | – | 1% | |
| | **Endocardial disease** *(percent incidence; number not reported)* | | | | | |
| | M | 1% | – | – | 3% | |
| | F | 0% | – | – | 0% | |
| | **Absolute heart weight; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −6% | −2% | −5% | |
| | F | 0% | 13% | 3% | 15% | |
| | **Relative heart-to-body weight; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −2% | 4% | 1% | |
| | F | 0% | 23% | 13% | 27% | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Absolute heart weight; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 3% | −2% | −2% | 1% |
| | F | 0% | −1% | 0% | −4% | −3% |
| | **Relative heart-to-body weight; 104 wks (percent change compared to control)** | | | | | |
| | M | 0% | 2% | 6% | 0% | 22% |
| | F | 0% | −2% | 3% | −1% | 15% |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 7% | 7% |
| | F | 0% | – | – | – | 0% | 0% |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| **Experiment 1**: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | **Relative heart weight** *(percent change compared to control)* | | | | | |
| | M | 0% | – | – | – | 6% | 0% |
| | F | 0% | – | – | – | −4% | 0% |
| **Experiment 2**: 0, 40, 60, or 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[b]<br>Diet<br>13 wks | Doses | 0 | 80 | 160 | 320 | |
| | **Focal myocardial degeneration** *(incidence)* | | | | | |
| | M** | 0/10 | – | – | 5/10* | |
| | F*** | 0/11 | – | – | 2/11 | |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 0% | 0% | 8% | |
| | F | 0% | 0% | 0% | 8% | |
| | **Relative heart-to-body weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 0% | −2% | 6% | |
| | F | 0% | 0% | −2% | 2% | |
| | **Includes one affected and three unaffected animals that died prematurely.<br>***Includes one unaffected animal that died prematurely. | | | | | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Focal myocardial degeneration** *(incidence)* | | | | | | |
| | M | 3/10 | – | – | – | – | 1/10 |
| | F | 2/10 | – | – | – | – | 6/10 |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 0% | −8%* |
| | F | 0% | – | – | – | −6% | −11%* |
| | **Relative heart-to-body weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 3% | 0% |
| | F | 0% | – | – | – | −3% | −8% |
| | **Relative heart-to-brain weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −4% | −10%* |
| | F | 0% | – | – | – | −5% | −11%* |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size | No cardiac effects were observed (microscopic examination of heart was performed in randomly selected F2 animals).<br>Heart weight data were reported only for F2 generation controls, 5 and 16 mg/kg-day groups. | | | | | | |
| | **Doses** | **0** | **5** | **16** | **50** | | |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | | |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | F2 M | 0% | | 3.2% | −6.5% | | – |
| | F2 F | 0% | | 15% | −3.7% | | – |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **Cardiomyopathy** *(incidence)* | | | | | | |
| | M | 2/10 | – | – | – | – | 3/8 |
| | F | 0/10 | – | – | – | – | 1/6 |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −2% | −7% | −1% | 1% | 11% |
| | F | 0% | −2% | 0% | 8% | 7% | 6% |
| | **Relative heart-to-body weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 4% | 2% | 1% | −1% | 8% |
| | F | 0% | −2% | −7% | −6% | −9% | −16%* |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[c]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | All animals in the 300 and 600 mg/kg-d groups died prior to study termination. | | | | | | |
| | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Chronic focal myocarditis** *(incidence)* | | | | | | |
| | M | 8/30 | 8/10 | 6/10 | 1/10 | 1/10 | 0/10 |
| | F | 8/30 | 3/10 | 1/10 | 1/10 | 1/10 | 1/9 |
| | **Absolute heart weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −2% | −10% | −15% | – | – |
| | F | 0% | −3% | 0% | −5% | – | – |
| | **Relative heart-to-body weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 2% | −4% | 3% | – | – |
| | F | 0% | −2% | 0% | −3% | – | – |
| Von Oettingen et al. (1949)<br>Rats (sex/strain not specified); 20/group<br>Purity and particle size not specified<br>0, 15, 25, or 50 mg/kg-d<br>Diet<br>3 mo | The study authors reported that there were no cardiac effects (microscopic examination of the heart was performed in all rats; data were not shown). | | | | | | |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food | Doses | 0 | | 0.1 | 1 | | 10 |
| | **Focal hyalinization of the heart** *(incidence)* | | | | | | |
| | M | 0/3 | | – | – | | 0/3 |
| | F | 0/3 | | – | – | | 1/3 |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| 0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | **Absolute heart weight** *(percent change compared to control)* | | | | |
| | M | 0% | – | – | 31% |
| | F | 0% | – | – | 5.7% |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 0.1 | 1 | 10 |
| | **Myocarditis** *(percent change compared to control)* | | | | |
| | M | 1/3 | – | – | 1/3 |
| | F | 0/3 | – | – | 0/3 |
| | **Absolute heart weight** *(percent change compared to control)* | | | | |
| | M | 0% | 7% | −1% | 5% |
| | F | 0% | 10% | 12% | −12% |
| *Immune effects* | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2−98.7% pure, with 3−10% HMX as contaminant; 83−89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | No immune effects were observed with routine hematology, clinical chemistry, or histopathology evaluations. | | | | |
| | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **WBC count; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −13% | −8% | −16% | −30% |
| | F | 0% | 12% | 39%* | 28% | 0% |
| | **Absolute spleen weight; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 24% | 31% | −10% | −28% |
| | F | 0% | 4% | 15% | −17% | 16% |
| | **Relative spleen weight; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 26% | 32% | −11% | −21% |
| | F | 0% | 4% | 15% | −17% | 44% |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | **Doses** | **0** | **1.0** | **3.1** | **10** |
| | **WBC count; 104 wks** *(percent change compared to control)* | | | | |
| | M | 0% | −13% | −22%* | −34%* |
| | F | 0% | 5% | −32%* | −12% |
| | **Absolute spleen weight; 104 wks** *(percent change compared to control)* | | | | |
| | M | 0% | −11% | −16% | −4% |
| | F | 0% | 58% | 8% | 37% |
| | **Relative spleen weight; 104 wks** *(percent change compared to control)* | | | | |
| | M | 0% | −11% | −14% | 1% |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| | F | 0% | 77% | 19% | 55% | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | No immune effects were observed with routine hematology, clinical chemistry and histopathology evaluations. | | | | | |
| | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **WBC count; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −11% | 103%[d] | 184%[d] | 15% |
| | F | 0% | 7% | 12% | 354%[d] | 251%[d] |
| | **Absolute spleen weight; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 5% | −10% | −32% | −49% |
| | F | 0% | −28% | −44% | −35% | 17% |
| | **Relative spleen weight; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 9% | 4% | −29% | −38% |
| | F | 0% | −34% | −45% | −36% | 9% |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10–12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>**Experiment 1**: 0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 18% | 13% |
| | F | 0% | – | – | – | −2% | −8% |
| | **Relative spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 24% | 14% |
| | F | 0% | – | – | – | −3% | −5% |
| **Experiment 2**: 0, 40, 60, 80 mg/kg-d for 2 wks followed by 0, 320, 160, or 80 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[b]<br>Diet<br>13 wks | Doses | 0 | 80 | 160 | 320 | |
| | **WBC count** *(percent change compared to control)* | | | | | |
| | M | 0% | −27% | −12% | 30% | |
| | F | 0% | −17% | 3% | −3% | |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 17% | 0% | −17% | |
| | F | 0% | −22% | 0% | 0% | |
| | **Relative spleen weight** *(percent change compared to control)* | | | | | |
| | M | 0% | 25% | 5% | 0% | |
| | F | 0% | −12% | 0% | −3% | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | −12% | 7% |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| 88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | F | 0% | – | – | – | 17% | 30% |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 2% | −4% |
| | F | 0% | – | – | – | −10% | −12%* |
| | **Relative spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 5% | 5% |
| | F | 0% | – | – | – | −8% | −8% |
| Cholakis et al. (1980)<br>Rats, CD, two-generation study; F0: 22/sex/group; F1: 26/sex/group; F2: 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>F0 and F1 parental animals: 0, 5, 16, or 50 mg/kg-d<br>Diet<br>13 wks | No immune effects were observed upon routine histopathology evaluation. | | | | | | |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | No effects were observed on thymus or spleen histology, red and white blood cell populations, or lymphocyte populations. | | | | | | |
| | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | −5% | −12% | −7% | 1% | −3% |
| | F | 0% | 22% | 45% | 12% | 52% | 29% |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −3% | −6% | 3% | 1% | 5% |
| | F | 0% | 1% | 8% | 23%* | 17%* | 24%* |
| | **Relative spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | 3% | 4% | 7% | −1% | 2% |
| | F | 0% | 1% | 0% | 6% | −1% | −2% |
| | **Absolute thymus weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −1% | 3% | −10% | −12% | −25% |
| | F | 0% | −7% | 12% | 19% | 32% | 19% |
| | **Relative thymus weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −1% | 3% | −10% | −12% | −25% |
| | F | 0% | −7% | 4% | 4% | 12% | −6% |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[c] | Data were not reported for rats in the 300 or 600 mg/kg dose groups because all of the rats died before the 13-wk necropsy. | | | | | | |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | 4% | 7% | 15% | – | – |
| | F | 0% | 23%* | 24%* | 62%* | – | – |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −11% | −16% | −34% | – | – |
| | F | 0% | 2% | 12% | 0% | – | – |
| | **Relative spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | −9% | −12% | −21% | – | – |
| | F | 0% | 2% | 12% | 3% | – | – |
| Von Oettingen et al. (1949)<br>Rats, sex/strain not specified, 20/group<br>90–97% pure, with 3–10% HMX; particle size not specified<br>0, 15, 25, or 50 mg/kg-d<br>Diet<br>3 mo | Doses | 0 | | 15 | 25 | | 50 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | | −30% | 7% | | −6% |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Doses | 0 | | 0.1 | 1 | | 10 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | | 5% | 2% | | −19% |
| | F | 0% | | −2% | 24% | | 6% |
| | **Absolute spleen weight** *(percent change compared to control)* | | | | | | |
| | M | 0% | | – | – | | 123% |
| | F | 0% | | – | – | | −11% |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | | 0.1 | 1 | | 10 |
| | **WBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | | −32% | 0% | | −3% |
| | F | 0% | | −38% | −1% | | −41% |
| *Gastrointestinal effects* | | | | | | | |

| Reference and study design | Results |
|---|---|
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | No gastrointestinal tract effects were observed as clinical signs or on gross pathology or histopathology examination. |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | No gastrointestinal tract effects were observed as clinical signs or on gross pathology or histopathology examination. |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | No gastrointestinal tract effects were observed on gross pathology or histopathology examination. Increased salivation and blood stains around the mouth were noted (affected doses and incidences were not reported); it is not clear whether these effects occurred in animals also experiencing convulsions. |
| Von Oettingen et al. (1949)<br>Rats (sex/strain not specified); 20/group<br>90–97% pure, with 3–10% HMX; particle size not specified<br>0, 15, 25, or 50 mg/kg-d<br>Diet<br>3 mo | Congestion of the gastrointestinal tract was observed in 50 and 100 mg/kg-d rats that also exhibited mortality (40%) and severe neurotoxicity. |
| Martin and Hart (1974)<br>Monkeys (Cynomolgus or Rhesus); 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 d | Vomiting was observed more frequently in the 1 and 10 mg/kg-d groups compared to the control or 0.1 mg/kg-d groups, although some episodes occurred during the intubation procedure. |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 grams of dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Some nausea and vomiting were reported (incidences and affected dose groups were not reported). |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| *Hematological effects* | | | | | | |
| Lish et al. (1984); Levine et al. (1984)<br>Mice, B6C3F$_1$, 85/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 1.5, 7.0, 35, or 175/100 mg/kg-d (high dose reduced to 100 mg/kg-d in wk 11 due to excessive mortality)<br>Diet<br>24 mo | Doses | 0 | 1.5 | 7.0 | 35 | 175/100 |
| | **RBC count; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −4% | 3% | −3% | 14% |
| | F | 0% | 4% | −7% | 5% | 3% |
| | **Hemoglobin; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −6% | 3% | −5% | 9% |
| | F | 0% | 2% | −7% | 3% | 1% |
| | **Hematocrit; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | −4% | 3% | −4% | 9% |
| | F | 0% | 3% | −6% | 3% | 1% |
| | **Platelets; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 33% | 9% | 21% | 27% |
| | F | 0% | −14% | −7% | 1% | 5% |
| Hart (1976)<br>Rats, Sprague-Dawley, 100/sex/group<br>Purity and particle size not specified<br>0, 1.0, 3.1, or 10 mg/kg-d<br>Diet<br>2 yrs | Doses | 0 | 1.0 | 3.1 | 10 | |
| | **RBC count; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 3% | 7% | −2% | |
| | F | 0% | −14% | 7% | 2% | |
| | **Reticulocyte count; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 250%[c] | 500%*[c] | 850%*[c] | |
| | F | 0% | 180%*[c] | −40% | 20% | |
| | **Hemoglobin; 104 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 3% | 4% | 0% | |
| | F | 0% | −1% | 1% | −2% | |
| Levine et al. (1983); Thompson (1983)<br>Rats, F344, 75/sex/group; interim sacrifices (10/sex/group) at 6 and 12 mo<br>89.2–98.7% pure, with 3–10% HMX as contaminant; 83–89% of particles <66 µm<br>0, 0.3, 1.5, 8.0, or 40 mg/kg-d<br>Diet<br>24 mo | Doses | 0 | 0.3 | 1.5 | 8.0 | 40 |
| | **Hemoglobin levels; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 6% | 6% | 3% | −13% |
| | F | 0% | −5% | 1% | −9% | −14% |
| | **RBC count; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 5% | 2% | −1% | −9% |
| | F | 0% | −2% | 2% | −9% | −13% |
| | **Platelet count; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 6% | −4% | −10% | −7% |

| Reference and study design | Results | | | | | |
|---|---|---|---|---|---|---|
| | F | 0% | 14% | −4% | 5% | 22% |
| | **Hematocrit; 105 wks** *(percent change compared to control)* | | | | | |
| | M | 0% | 5% | 5% | 2% | −7% |
| | F | 0% | −5% | 0% | −8% | −12% |
| Cholakis et al. (1980)<br>Mice, B6C3F$_1$, 10−12/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 80, 60, or 40 mg/kg-d for 2 wks followed by 0, 80, 160, or 320 mg/kg-d (TWA doses of 0, 79.6, 147.8, or 256.7 mg/kg-d for males and 0, 82.4, 136.3, or 276.4 mg/kg-d for females)[b]<br>Diet<br>13 wks | Doses | 0 | 80 | 160 | 320 | |
| | **RBC count** *(percent change compared to control)* | | | | | |
| | M | 0% | −5% | −12%* | −2% | |
| | F | 0% | −10% | −1% | 1% | |
| | **Reticulocytes** *(percent change compared to control)* | | | | | |
| | M | 0% | −36% | −13% | 15% | |
| | F | 0% | 21% | 25% | −19% | |
| | **Hematocrit** *(percent change compared to control)* | | | | | |
| | M | 0% | −1% | −6% | 0% | |
| | F | 0% | −8% | 2% | 1% | |
| | **Hemoglobin** *(percent change compared to control)* | | | | | |
| | M | 0% | −2% | −7%* | −3% | |
| | F | 0% | −5% | 4% | 1% | |
| | **Platelets** *(percent change compared to control)* | | | | | |
| | M | 0% | 33% | 28% | 22% | |
| | F | 0% | 3% | 9% | 39% | |
| Cholakis et al. (1980)<br>Rats, F344, 10/sex/group<br>88.6% pure, with 9% HMX and 2.2% water as contaminants; ~200 µm particle size<br>0, 10, 14, 20, 28, or 40 mg/kg-d<br>Diet<br>13 wks | Doses | 0 | 10 | 14 | 20 | 28 | 40 |
| | **RBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 3% | −1% |
| | F | 0% | – | – | – | −1% | −7% |
| | **Hemoglobin** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 2% | −1% |
| | F | 0% | – | – | – | −1% | −1% |
| | **Platelet** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 11% | 16%* |
| | F | 0% | – | – | – | −23% | −13% |
| | **Reticulocytes** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 26% | 76%* |
| | F | 0% | – | – | – | −2% | 17% |

| Reference and study design | Results | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Hematocrit** *(percent change compared to control)* | | | | | | |
| | M | 0% | – | – | – | 3% | 0% |
| | F | 0% | – | – | – | 0% | −2% |
| Crouse et al. (2006)<br>Rats, F344, 10/sex/group<br>99.99% pure<br>0, 4, 8, 10, 12, or 15 mg/kg-d<br>Gavage<br>90 d | Doses | 0 | 4 | 8 | 10 | 12 | 15 |
| | **RBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | 1% | −7% | −2% | −4% | −5% |
| | F | 0% | 3% | 3% | −1% | 2% | −2% |
| | **Hemoglobin** *(percent change compared to control)* | | | | | | |
| | M | 0% | −1% | −5% | 0% | −1% | −6% |
| | F | 0% | 2% | 4% | −1 | 4% | −4% |
| | **Platelet count** *(percent change compared to control)* | | | | | | |
| | M | 0% | 21% | 11% | 13% | −8% | 34% |
| | F | 0% | 6% | 40% | 47% | 34% | −36% |
| | **Hematocrit** *(percent change compared to control)* | | | | | | |
| | M | 0% | 2% | −5% | 0% | −1% | −4% |
| | F | 0% | 3% | 4% | 0% | 4% | −2% |
| Levine et al. (1981a);Levine et al. (1990); Levine et al. (1981b)[c]<br>Rats, F344, 10/sex/group; 30/sex for control<br>84.7 ± 4.7% purity, ~10% HMX, median particle diameter 20 µm, ~90% of particles ≤ 66 µm<br>0, 10, 30, 100, 300, or 600 mg/kg-d<br>Diet<br>13 wks | Data were not reported for rats in the 300 or 600 mg/kg dose groups because all of the rats died before the 13-wk necropsy. | | | | | | |
| | Doses | 0 | 10 | 30 | 100 | 300 | 600 |
| | **Hematocrit** *(percent change compared to control)* | | | | | | |
| | M | 0% | −2% | −1% | −5% | – | – |
| | F | 0% | 0% | −4% | −7% | – | – |
| | **Hemoglobin** *(percent change compared to control)* | | | | | | |
| | M | 0% | −3% | −1% | −6% | – | – |
| | F | 0% | 0% | −4% | −8%* | – | – |
| | **RBC count** *(percent change compared to control)* | | | | | | |
| | M | 0% | −2% | −2% | −5% | – | – |
| | F | 0% | −1% | −4% | −5% | – | – |
| | **Reticulocytes** *(percent change compared to control)* | | | | | | |
| | M | 0% | −4% | 10% | 28% | – | – |
| | F | 0% | 9% | 73% | 71% | – | – |
| Von Oettingen et al. (1949)<br>Rats, sex/strain not specified, 20/group<br>90–97% pure, with 3–10% HMX; particle size not specified | Doses | 0 | | 15 | 25 | | 50 |
| | **RBC count** *(percent change compared to control)* | | | | | | |
| | M + F | 0% | | −23% | −12% | | −14% |

| Reference and study design | Results | | | | |
|---|---|---|---|---|---|
| 0, 15, 25, or 50 mg/kg-d<br>Diet<br>3 mo | **Hemoglobin** *(percent change compared to control)* | | | | |
| | M + F | 0% | −25% | −7% | −11% |
| Hart (1974)<br>Dogs, Beagle, 3/sex/group<br>Pre-mix with ground dog chow containing 20 mg RDX/g-chow, 60 g dog food<br>0, 0.1, 1, or 10 mg/kg-d<br>Diet<br>90 d | Doses | 0 | 0.1 | 1 | 10 |
| | **RBC count** *(percent change compared to control)* | | | | |
| | M | 0% | −3% | 3% | 2% |
| | F | 0% | 13% | 7% | 11% |
| | **Reticulocyte count** *(percent change compared to control)* | | | | |
| | M | 0% | −66% | 0% | −50% |
| | F | 0% | −17% | −50% | 0% |
| | **Hematocrit** *(percent change compared to control)* | | | | |
| | M | 0% | −4% | 2% | 0% |
| | F | 0% | 6% | 1% | 7% |
| | **Hemoglobin** *(percent change compared to control)* | | | | |
| | M | 0% | 5% | −2% | 0% |
| | F | 0% | 8% | −2% | 8% |
| Martin and Hart (1974)<br>Monkeys, Cynomolgus or Rhesus, 3/sex/group<br>Purity of test material not specified<br>0, 0.1, 1, or 10 mg/kg-d<br>Gavage<br>90 ds | Histopathological examination revealed increased numbers of degenerate or necrotic megakaryocytes in all bone marrow sections. | | | | |
| | Doses | 0 | 0.1 | 1 | 10 |
| | **RBC count** *(percent change compared to control)* | | | | |
| | M | 0% | −3% | 2% | −3% |
| | F | 0% | 0% | −1% | 2% |
| | **Reticulocyte count** *(percent change compared to control)* | | | | |
| | M | 0% | −33% | −50% | −50% |
| | F | 0% | −18% | −36% | 45% |
| | **Hematocrit** *(percent change compared to control)* | | | | |
| | M | 0% | −7% | −4% | −1% |
| | F | 0% | 10% | 7% | 3% |
| | **Hemoglobin** *(percent change compared to control)* | | | | |
| | M | 0% | −10% | −8% | −6% |
| | F | 0% | 6% | 6% | 3% |

*Statistically significantly different compared to the control, as determined by study authors ($p < 0.05$).
[a]Incidence counts exclude individuals from which blood was obtained via the orbital sinus.
[b]Doses were calculated by the study authors.

[c]Levine et al. (1981a) is a laboratory report of a 13-week study of RDX in F344 rats; two subsequently published papers (Levine et al., 1990; Levine et al., 1981b) present subsets of the data provided in the full laboratory report.
[d]Standard deviations accompanying the mean response in a given dose group were high, suggesting uncertainty in the accuracy of the reported percent change compared to control.

***Summary of Other Toxicity Data***

Effects on the eyes and the musculoskeletal, cardiovascular, immune, and gastrointestinal systems have been reported in some studies. EPA concluded that the evidence does not support these effects as a potential human hazard of RDX exposure.

## 1.2. INTEGRATION AND EVALUATION

### 1.2.1. Effects Other Than Cancer

The majority of evidence for the health effects of RDX comes from oral toxicity studies. The available health effects literature does not support identification of hazards by the inhalation route of exposure. Three epidemiological studies that document possible inhalation exposure are limited by various study design features, including inability to distinguish exposure to TNT (associated with liver and hematological system toxicity), uncertainty in identifying exposure levels, small sample sizes, and inadequate reporting. The single animal inhalation study identified in the literature search had deficiencies that precluded its inclusion in this assessment (see Literature Search Strategy | Study Selection and Evaluation).

The strongest evidence for hazards following exposure to RDX is for nervous system effects. A human occupational study (Ma and Li, 1992) describes memory impairment and visual-spatial decrements, and several case reports provide additional evidence of associations between exposure to RDX and seizures and convulsions (Kasuske et al., 2009; Küçükardali et al., 2003; Testud et al., 1996b; Testud et al., 1996a; Woody et al., 1986 and others, see Appendix C.3). Other nervous system effects identified in human case reports include dizziness, headache, confusion, and hyperirritability. Evidence from toxicity studies in multiple animal species involving chronic, subchronic and gestational exposures is consistent with the effects seen in humans. Effects included dose-related increases in seizures and convulsions, as well as observations of tremors, hyperirritability, hyper-reactivity, and other behavioral changes (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Von Oettingen et al., 1949). In a number of these studies, death occurred at RDX doses that induced nervous system effects. Crouse et al. (2006), a study designed to more systematically record nervous system effects, reported that pre-term deaths occurred earlier in the higher-dose groups and in almost all cases, deaths were preceded by neurotoxic signs such as tremors and convulsions. The strength of a direct association between mortality and nervous system effects is less clear in most of the earlier studies because the frequency of clinical observations may have been insufficient to observe seizures prior to death.

Induction of convulsions and seizures appears to be more strongly correlated with dose than with duration of exposure. It is unclear if nervous system effects increased in severity (e.g., from behavioral change to seizures and convulsions) with increasing dose because many of the studies that reported more subtle neurobehavioral changes did not provide detailed dose-response information, and the majority of studies were not designed to capture this information. Additional

support for an association between RDX exposure and nervous system effects comes from consistent evidence of neurotoxicity across taxa, including several species of wildlife (Quinn et al., 2013; Garcia-Reyero et al., 2011; McFarland et al., 2009; Gogal et al., 2003). Although the MOA is unknown, the association between RDX and neurological effects is biologically plausible, with studies demonstrating a correlation between blood and brain concentrations of RDX and the time of seizure onset (Williams et al., 2011; Bannon et al., 2009). Additionally, the affinity of RDX for the picrotoxin convulsant site of the $GABA_A$ channel suggests that the resulting disinhibition could lead to the onset of seizures (Williams et al., 2011). EPA identified nervous system effects as a human hazard of RDX exposure.

Evidence for kidney and other urogenital toxicity is more limited than evidence for neurotoxicity. Increased relative kidney weight was observed in male and female mice (Lish et al., 1984), and histopathological changes in the urogenital system (including suppurative prostatitis) were reported in male rats exposed to RDX in the diet for 2 years (Levine et al., 1983). Similar histopathological changes of the urogenital system were not observed in mice, and no other rat studies of similar duration that examined the prostate were available. Among the lesions identified in the rat, the incidence of suppurative prostatitis is considered a marker for RDX-related urogenital effects. The plausibility of a MOA that shares a common molecular initiating event (binding to the $GABA_A$ receptor convulsant-site) with the neurotoxic effects of RDX increases support for an association between RDX exposure and kidney and other urogenital effects. EPA identified the urogenital system as a potential human hazard of RDX exposure.

Evidence for male reproductive toxicity comes from the finding of testicular degeneration in male $B6C3F_1$ mice chronically exposed to RDX in the diet (Lish et al., 1984) in the only mouse study conducted of that duration (24 months). The effect was noted by study authors at both the penultimate and high dose tested in the study. However, studies in different rat strains did not consistently report testicular effects. Although the available data are limited, given the dose-related findings of mouse testicular degeneration, EPA identified suggestive evidence of male reproductive effects as a potential human hazard of RDX exposure.

Evidence for developmental toxicity and liver toxicity was more limited than that for the endpoints discussed above. In animal studies, embryotoxicity and other developmental effects were observed only at doses associated with maternal mortality (Angerhofer et al., 1986; Cholakis et al., 1980). Evidence for hepatic effects comes from observations of increases (generally dose-related) in liver weight in some chronic and subchronic oral animal studies (Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Hart, 1976). However, these elevations in liver weight were not accompanied by RDX-related histopathological changes in the liver or increases in serum liver enzymes. In addition, interpretation of liver weight changes in the mouse bioassay by Lish et al. (1984) is complicated by the relatively high incidence of liver tumors in this study. EPA concluded that evidence does not support developmental toxicity or liver

effects as potential human hazards of RDX exposure. Thus, these effects were not considered further for dose-response analysis and the derivation of reference values.

### 1.2.2. Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the database for RDX provides "suggestive evidence of carcinogenic potential" based on the finding of statistically significant trends for hepatocellular adenomas or carcinomas and alveolar/bronchiolar adenomas or carcinomas in female, but not male, B6C3F$_1$ mice (Lish et al., 1984). This is further supported by the finding of a statistically significant trend for hepatocellular carcinomas in male, but not female, F344 rats (Levine et al., 1983) exposed to RDX in the diet for two years. On the other hand, there was no evidence of carcinogenicity in Sprague-Dawley rats in a 2-year dietary study of RDX (Hart, 1976). No human studies are available to assess the carcinogenic potential of RDX.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) emphasizes the importance of weighing the totality of evidence in reaching conclusions about the human carcinogenic potential of agents under evaluation. Information taken into consideration in weighing the evidence for the human carcinogenic potential of RDX includes the magnitude of response in rats, availability of a PWG reevaluation of tumors, and potential differences in test material across studies.

The incidence of male rat liver carcinomas as reported by Levine et al. (1983) showed a positive trend with dose (based on statistical analysis conducted for this review), thus supporting the positive finding of liver tumors in female mice. However, as discussed in Section 1.1.5, the association of liver tumors in rats with RDX exposure was judged not to be strong for several reasons, including the small numbers of carcinomas observed across the study and the low survival rate in the high-dose group that reduces confidence that the final incidence in that group accurately reflects lifetime cancer incidence. A PWG reevaluation of rat liver tumors has not been conducted.

The weight of evidence of carcinogenicity also took into consideration the lack of carcinogenic response in the two-year bioassay in the Sprague-Dawley rat (Hart, 1976). The incidence of liver tumors in the Hart (1976) study was not increased relative to controls at a dose of 10 mg/kg-day, a dose that fell in the range of doses in the Levine et al. (1983) study that showed a positive tumor trend.

A cancer descriptor may be applicable to a variety of potential data sets and represent points along a continuum of evidence (U.S. EPA, 2005a). The available evidence for RDX suggests that it could be considered a borderline case between two descriptors—"likely to be carcinogenic to humans" and "suggestive evidence of carcinogenic potential." One of the criteria identified in EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) that supports the likely descriptor is "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" may qualify as a likely carcinogen. Bioassay data provide evidence for RDX carcinogenicity in one sex of one species (female mouse), weaker evidence for carcinogenicity in one sex of a second species (male rat), and

evidence of tumors in two tissues (liver and lung); this evidence could be considered to meet the criteria for the "likely to be carcinogenic to humans" descriptor.

The "suggestive evidence of carcinogenic potential" descriptor is appropriate when the weight of evidence is suggestive of carcinogenicity, and a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a wide spectrum of evidence associated with varying levels of concern for carcinogenicity, including a positive cancer result in the only study on an agent, a single positive cancer result in an extensive database that includes negative studies in other species, or evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a positive conclusion.

In reviewing the carcinogenicity data for RDX, EPA considered that either descriptor is plausible, as the evidence for increased trends in tumor incidence in two tissues and possibly a second species raises a concern for carcinogenic effects in humans. However, in light of the determination that the association between RDX exposure and liver tumors in rats is not strong, and the lack of a carcinogenic response in male B6C3F$_1$ mice, female F344 rats, and Sprague-Dawley rats of both sexes, EPA concluded that there is "suggestive evidence of carcinogenic potential" for RDX.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. Information available on the carcinogenic effects of RDX via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of RDX via the inhalation and dermal routes in humans or animals is not available. Based on the observation of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, there is "suggestive evidence of carcinogenic potential" following exposure to RDX by all routes of exposure.

### 1.2.3. Susceptible Populations and Lifestages for Cancer and Noncancer Outcomes

Susceptibility refers to factors such as lifestage, genetics, sex, and health status that may predispose a group of individuals to greater response to an exposure. This greater response could be achieved either through differences in exposure to the chemical underlying toxicokinetic and toxicodynamic differences between susceptible and other populations. Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Lifestage, and in particular childhood susceptibility, has not been observed in human or animal studies of RDX toxicity. Reproductive and developmental toxicity studies did not identify effects in offspring at doses below those that also caused maternal toxicity (Angerhofer et al., 1986; Cholakis et al., 1980). Transfer of RDX from dam to the fetus during gestation has been reported, and the presence of RDX in the milk of dams administered 6 mg/kg-day by gavage has been documented (Hess-Ruth

et al., 2007). Because RDX is neurotoxic in adult animals, evidence of gestational transfer of RDX to the developing organism, along with the presence of RDX in milk, suggests that the nervous system may be a target in the developing organism; however, developmental neurotoxicity studies of RDX have not been conducted. Limited data suggest that male laboratory animals may be more susceptible to noncancer toxicity associated with RDX exposure. While no sex-based differences in neurotoxicity were observed, urogenital effects have been observed in males at lower doses than in females (Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980), suggesting a possible sex-based difference in susceptibility to RDX toxicity. There is limited evidence that CYP450 or similar enzymes are involved in the metabolism of RDX (Bhushan et al., 2003), indicating a potential for genetic polymorphisms in these metabolic enzymes to affect susceptibility to RDX. This susceptibility may also be influenced by differential expression of these enzymes during development. Individuals with epilepsy or other seizure syndromes, and in particular those that have their basis in genetic mutation to $GABA_A$ receptors, may represent another group that may be susceptible to RDX exposure. However, there is currently no information to support predictions of how genetic polymorphisms may affect susceptibility.

# 2. DOSE-RESPONSE ANALYSIS

## 2.1. ORAL REFERENCE DOSE FOR EFFECTS OTHER THAN CANCER

The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a no-observed-adverse-effect level (NOAEL), lowest-observed-adverse-effect level (LOAEL), or the 95% lower bound on the benchmark dose (BMDL), with uncertainty factors (UFs) generally applied to reflect limitations of the data used.

### 2.1.1. Identification of Studies and Effects for Dose-Response Analysis

Human studies are generally preferred over animal studies as the basis for a reference value when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure. The available epidemiological studies of worker populations exposed to RDX examined the relationship between certain health endpoints and inhalation exposure; no epidemiological studies of ingested RDX are available. Therefore, epidemiological studies could not be used for oral dose-response analysis and as the basis for the RfD. Multiple case reports provide some evidence of effects in humans associated with acute exposure to RDX; however, while case reports can support the identification of hazards associated with RDX exposure, data from case reports are inadequate for dose-response analysis and subsequent derivation of a chronic reference value because of short exposure durations and incomplete or missing quantitative exposure information.

As discussed in Section 1.2.1, based on findings from oral studies in experimental animals, EPA identified nervous system effects as a human hazard of RDX exposure, and effects on the urogenital system (including the kidney) as a potential human hazard of RDX exposure. EPA also identified suggestive evidence of male reproductive effects as a potential human hazard of RDX exposure. Experimental animal studies within each health effect category were evaluated using general study quality considerations discussed in Section 6 of the Preamble and in the section on Literature Search Strategy | Study Selection and Evaluation to help inform the selection of studies from which to derive oral reference values. Rationales for selecting the studies and effects to represent each of these hazards are summarized below.

#### *Nervous System Effects*

Nervous system effects following oral exposure to RDX, including convulsions, seizures, and hyper-reactivity, were observed in multiple studies in rats, mice, monkeys, and dogs. Only three

studies reported data on the incidence of nervous system findings—Crouse et al. (2006), Cholakis et al. (1980), and Martin and Hart (1974). Two of these—Crouse et al. (2006) and Cholakis et al. (1980)—were selected for dose-response analysis.

Crouse et al. (2006) reported a dose-related increase in convulsions and tremors in both male and female F344 rats following a 90-day oral (gavage) exposure to RDX. Additionally, Crouse et al. (2006) observed that for all the dose groups where unscheduled deaths were recorded, mortality was strongly associated with seizures or convulsions. This study used a test material of high purity (99.99% RDX), six dose groups (including the control) that provided good resolution of the dose-response curve, and relatively low doses that still provided adequate responses. Cholakis et al. (1980) reported a dose-related increase in convulsions in a developmental toxicity study, with convulsions observed at a dose as low as 2 mg/kg-day RDX on GDs 6–19. Because evidence of nervous system effects was observed in this study at a relatively low dose, this study was also selected for dose-response analysis.

The study in monkeys by Martin and Hart (1974) was not selected for dose-response analysis. This study provided supporting evidence of nervous system effects (trembling, shaking, ataxia, and hyperactive reflexes) with 66% incidence at the high dose of 10 mg/kg-day; however, this study was not selected for dose-response analysis because it used small group sizes (n = 3/sex) and the exposures were relatively variable or uncertain (e.g., purity of the test material was not specified, and reported emesis in some animals likely influenced the amount of dose received).

Other chronic and subchronic studies reported nervous system effects as clinical observations (Angerhofer et al., 1986; Lish et al., 1984; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Von Oettingen et al., 1949), but without incidence data. As discussed in Section 1.1.1, these studies did not systematically monitor or evaluate nervous system effects induced by RDX, leading to possible underestimates of incidence of such effects. As such, there is some uncertainty associated with identification of NOAELs and LOAELs for nervous system effects from these studies. Further, these studies reported convulsions and other indications of nervous system effects at doses higher than the doses at which effects were observed in Cholakis et al. (1980), i.e., ≥2 mg/kg-day, and Crouse et al. (2006), i.e., ≥8 mg/kg-day.

***Kidney and Other Urogenital Effects***

Effects on kidney and other urogenital system endpoints included changes in kidney weight and histopathological findings in the kidney, bladder, and prostate in experimental animals exposed orally to RDX. As discussed in Section 1.1.3, kidney weight changes across experimental animal studies were not consistent and were difficult to interpret; therefore kidney weight data sets were not selected for quantitative analysis.

Histopathological changes in the urogenital system were reported in a 2-year study in F344 rats by Levine et al. (1983) and in a 13-week study in B6C3F$_1$ mice by Cholakis et al. (1980). Histopathological changes of the kidney and bladder (medullary papillary necrosis, suppurative pyelitis, uremic mineralization, and luminal distention and cystitis of the urinary bladder) were

observed by Levine et al. (1983) in high-dose (40 mg/kg-day) males. The incidence of suppurative prostatitis, considered to be a marker for the broader range of urogenital effects in these animals, showed a dose-related trend beginning at doses below 40 mg/kg-day (see Section 1.1.3). Therefore, suppurative prostatitis was selected for dose-response modeling as a sensitive measure of RDX effects on the urogenital system.

Cholakis et al. (1980) examined the kidney for histopathological changes in control and high-dose (320 mg/kg-day) mice only. Because incidence data from only a single high-dose group was available, this study was not selected for dose-response analysis.

***Male Reproductive Toxicity***

Male reproductive effects were identified in mice following chronic administration of RDX in the diet. Lish et al. (1984) observed an increased incidence of testicular degeneration in mice given RDX in diet for two years compared to controls. The response was shown to be dose-related and was selected for dose-response modeling. Changes in other reproductive outcomes were not dose-related or consistently observed across studies, and therefore were not considered for dose-response modeling.

### 2.1.2. Methods of Analysis

Benchmark dose (BMD) modeling and physiologically-based pharmacokinetic (PBPK) models were used in this assessment to estimate candidate points of departure (PODs) for the derivation of an RfD for RDX. The general approach for the estimation of PODs is presented in Figure 2-1 and described further below.

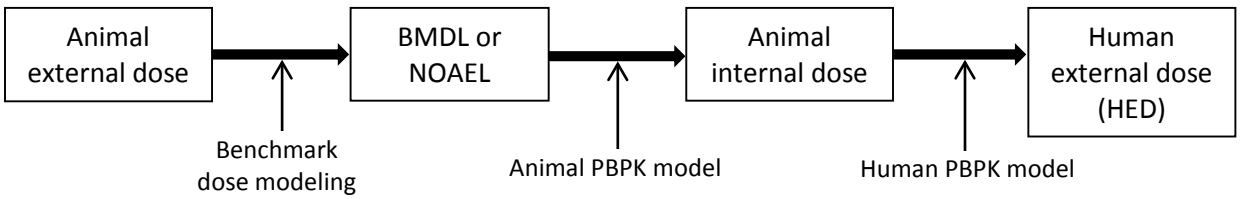



**Figure 2-1. Approach for dose-response analysis.**

No biologically based dose-response models are available for RDX. In this situation, EPA evaluates a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of the observed data. Consistent with this approach, EPA evaluated dose-response information with the models available in EPA's Benchmark Dose Software (BMDS, versions 2.4 and 2.5). EPA estimated the benchmark dose (BMD) and 95% lower confidence limit on the BMD (BMDL) using a benchmark response (BMR) selected for each effect. A summary of BMD modeling, including

selection of BMRs, for each of the health effect categories is provided below.

***Nervous System Effects***

Incidence data from Crouse et al. (2006) and Cholakis et al. (1980) were amenable to modeling. For Crouse et al. (2006), statistical analysis (Cochran-Mantel-Haenszel test) conducted by EPA indicated no significant difference in convulsion rates of male and female rats; thus, combined incidence data from male and female rats were used for modeling convulsion data from this study. A BMR of 1% extra risk for convulsions was used to address the relative severity of this endpoint; across the experimental animal database for RDX, convulsions and seizures were generally associated with mortality. In general, severe endpoints are not used as the basis of a noncancer risk value because of relatively high uncertainty in extrapolating to a level of exposure likely to be without appreciable risk. Less severe nervous system outcomes that precede convulsions and associated mortality would be preferred, but none were identified for RDX.

***Kidney/Urogenital and Male Reproductive Effects***

Incidence data on prostate effects as reported by Levine et al. (1983) and testicular degeneration as reported by Lish et al. (1984) were amenable to modeling. Cut-offs for the biological significance of these effects were not identified, and a BMR of 10% was applied under the assumption that it represents a minimally biologically significant degree of effect. Uncertainty in this characterization should be taken into account in comparisons with PODs from other effects.

***Human Extrapolation***

EPA guidance (U.S. EPA, 2011) advocates a hierarchy of approaches for deriving human equivalent doses (HEDs) from data in laboratory animals, with the preferred approach being physiologically-based toxicokinetic modeling. Other approaches can include using chemical-specific information in the absence of a complete physiologically-based toxicokinetic model. In lieu of either reliable chemical-specific models or data to inform the derivation of human equivalent oral exposures, a body weight scaling to the ¾ power (i.e., $BW^{3/4}$) approach is generally applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans for the purpose of deriving an oral RfD.

As described below, HEDs for candidate PODs for RDX were derived using PBPK models for endpoints selected from rat and mouse bioassays, and are compared in Table 2-1 to estimates derived from administered RDX dose.

**Table 2-1. Summary of derivation of PODs following oral exposure to RDX**

| Endpoint and reference (exposure duration/route) | Species/sex | Model[a] | BMR | BMD (mg/kg-d) | BMDL (mg/kg-d) | $POD_{HED}$ (mg/kg-d) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Administered dose[b] | RDX AUC[c] |
| Nervous system | | | | | | | |
| Convulsions Crouse et al. (2006) (90-d/gavage) | Male and female F344 rat, combined | Multistage 3° | 1% ER | 1.53 | 0.54 | 0.13 | 0.27 |
| Convulsions Cholakis et al. (1980) (GDs 6–19/gavage) | Female F344 rat | Quantal-linear | 1% ER | 0.18 | 0.12 | 0.03 | 0.06 |
| Kidney/urogenital system | | | | | | | |
| Prostate suppurative inflammation Levine et al. (1983) (2-yr/diet) | Male F344 rat | LogProbit | 10% ER | 1.67 | 0.47 | 0.11 | 0.23 |
| Male reproductive system | | | | | | | |
| Testicular degeneration Lish et al. (1984) (2-yr/diet) | Male B6C3$F_1$ mouse | LogProbit | 10% ER | 56.0 | 16.3 | 2.4 | 0.08 |

[a]For modeling details, see Appendix D.
[b]POD was convered to an HED using a standard DAF based on $BW^{3/4}$.
[c]POD was converted to an HED based on the equivalence of internal RDX dose (expressed as area under the curve [AUC] for RDX concentration in arterial blood) derived using PBPK models.

ER = extra risk

Physiologically-based pharmacokinetic models for RDX in rats, humans, and mice have been published (Sweeney et al., 2012a; Sweeney et al., 2012b; Krishnan et al., 2009) based on RDX-specific data. EPA evaluated and further developed these models for extrapolating doses from animals to humans (see Appendix C, Section C.2.5). As concluded in the MOA analyses for the various observed noncancer effects associated with RDX exposure, the available data are insufficient to establish any specific mode(s) of action for these effects, and there appears to be no clear evidence linking health effects with RDX-generated metabolites. In general, appropriately chosen internal dose metrics are expected to correlate more closely with toxic responses than external doses, for effects that are not occurring at the point of contact (Mclanahan et al., 2012).

Therefore, PBPK model-derived arterial blood concentration of RDX is considered a better dose-metric for extrapolation of health effects than administered dose when there is adequate confidence in the estimated value. The PBPK models for RDX were used to estimate the area under the curve (AUC) for RDX concentration in arterial blood, which represents the average blood RDX concentration for the exposure duration normalized to 24 hours.

It appears logical to use RDX concentration levels in the brain as the internal dose metric for analyzing convulsions as the health effect. Nevertheless, the blood concentration of RDX was preferred as the dose metric due to greater confidence in modeling this variable. This is because of the substantially greater number of measurements of RDX blood levels used in calibrating model parameters. Additionally, predictions of RDX concentrations in the brain are highly correlated with RDX blood concentrations because the brain compartment does not have absorption, metabolism, or elimination of RDX. It may also be noted that there is greater confidence in model estimates of blood AUC versus peak blood concentrations because, as discussed in Appendix C, Section C.2.5, the rate constant for oral absorption (KAS) is uncertain, and peak concentrations are more sensitive to variations in this parameter than average values. Furthermore, a more consistent dose-response for convulsions is observed in chronic studies than for the higher exposures in subchronic studies.

The rodent PBPK model was applied to the BMDLs generated from BMD modeling to determine the animal internal dose, expressed as the AUC of RDX blood concentration, and representing the cross-species toxicologically equivalent dose. The human PBPK model was then applied to derive the corresponding HEDs (see Figure 2-1). Because the AUC is linear with exposure level, at least in the exposure range of interest, the value of the HED would be the same whether the rat or mouse PBPK model is applied before or after BMD modeling is performed (i.e., the sequence of this calculation is immaterial for the RDX data).

HEDs were also calculated consistent with EPA guidance (U.S. EPA, 2011) using PODs (BMDLs or NOAELs) determined from administered RDX doses and employing a standard dosimetric adjustment factor (DAF) derived as follows:

$$DAF = (BW_a^{1/4}/BW_h^{1/4}),$$

where

$BW_a$ = animal body weight

$BW_h$ = human body weight

Using a $BW_a$ of 0.25 kg for rats and 0.035 kg for mice and a $BW_h$ of 70 kg for humans (U.S. EPA, 1988), the resulting DAFs for rats and mice are 0.24 and 0.15, respectively. Applying the DAF to the POD identified for effects in adult rats or mice yields a $POD_{HED}$ as follows (see Table 2-1):

$$POD_{HED} = \text{Laboratory animal dose (mg/kg-day)} \times DAF$$

Further details of the BMDL modeling, BMDS outputs, and graphical results for the best fit model for each dataset included in Table 2-1 can be found in Appendix D, Section D.1. Details of the PBPK model evaluation used for extrapolation from BMDL values can be found in Appendix C, Section C.2.5. Table 2-1 summarizes the results of the BMD modeling and the $POD_{HED}$ for each data set discussed above.

### 2.1.3. Derivation of Candidate Values

Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing. Because of relatively high confidence in the rat and human PBPK modeling, these models were used to derive reliable internal dose metrics for extrapolation. For datasets selected from the rat bioassays, the candidate RfDs were calculated assuming cross-species toxicological equivalence of the AUC of RDX blood concentration derived from the PBPK modeling. However, there were major uncertainties identified in the mouse PBPK modeling. Therefore, for endpoints selected from the mouse bioassay, the preferred approach for determining the candidate RfDs is that based on the administered dose of RDX extrapolated to humans using allometric $BW^{3/4}$ scaling. The evaluation of confidence in the PBPK model results is summarized in *Summary of confidence in PBPK models for RDX* in Appendix C, Section C.2.5.

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) (Section 4.4.5), and as described in the Preamble, five possible areas of uncertainty and variability were considered. An explanation follows.

An intraspecies uncertainty factor, $UF_H$, of 10 was applied to all PODs to account for potential differences in toxicokinetics and toxicodynamics in the absence of information on the variability of response in the human population following oral exposure to RDX.

An interspecies uncertainty factor, $UF_A$, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to all PODs to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between rodents and humans. For the testicular degeneration dataset from the mouse bioassay, a $UF_A$ of 3 was applied because $BW^{3/4}$ scaling is used to extrapolate oral doses from laboratory animals to humans. Although $BW^{3/4}$ scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's $BW^{3/4}$ guidance (U.S. EPA, 2011) recommends use of an uncertainty factor of 3. For datasets from the rat bioassays, a PBPK model was used to convert internal doses in rats to administered doses in humans. This reduces toxicokinetic uncertainty in extrapolating from the rat to humans, but does not account for interspecies differences due to toxicodynamics. A $UF_A$ of 3 was applied to account for this remaining toxicodynamic and any residual toxicokinetic uncertainty not accounted for by the PBPK model.

A subchronic to chronic uncertainty factor, $UF_s$, differs depending on the exposure duration. An $UF_s$ of 1 was applied to the POD values for kidney/urogenital effects and testicular degeneration derived from the 2-year bioassays in the rat (Levine et al., 1983) and mouse (Lish et al., 1984). POD

values for nervous system effects were derived from studies of subchronic duration or gestational exposure; a $UF_S$ of 3 was applied to these PODs. Typically, a $UF_S$ of 10 is applied to extrapolate results from a subchronic duration study in the absence of a chronic study based on the assumption that effects from a given compound would occur at approximately a 10-fold higher exposure level in a subchronic study than in a chronic study, if a chronic study were available (U.S. EPA, 2002). However, the available nervous system effects data for RDX support an $UF_S$ of less than 10. As discussed in Section 1.1.1, seizure induction appears to be more strongly correlated with dose level than with duration of exposure. In addition, the available empirical evidence from rodent bioassays provide support for an $UF_S$ no greater than 3. Dose levels associated with convulsions in chronic dietary studies of RDX are ≥35 mg/kg-day and are higher than doses that induced convulsions in the 14- and 90-day (gavage) studies that were used to derive candidate PODs for nervous system effects (i.e., 2 mg/kg-day in Cholakis et al. (1980) and 8 mg/kg-day in Crouse et al. (2006)) (also see Table 1-2 and Figure 1-1). Thus, the available RDX data for nervous system effects is consistent with the application of a $UF_S$ that is less than the default of 10.

A LOAEL to NOAEL uncertainty factor, $UF_L$, of 1 was applied to all POD values because the PDO was a BMDL. When the POD is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for benchmark dose modeling. In this case, the BMR for modeled endpoints was selected under the assumption that the BMR represents a minimal, biologically significant change for these effects.

A database uncertainty factor, $UF_D$, of 3 was applied to all POD values. The oral toxicity database for RDX includes subchronic and chronic toxicity studies in the rat and mouse, a two-generation reproductive toxicity study in the rat, developmental toxicity studies in the rat and rabbit, and subchronic studies (with study design limitations) in the dog and monkey. Deficiencies in the database related to neurobehavioral and neurodevelopmental testing were identified. The database for neurotoxicity is characterized primarily by observations of frank effects (convulsions). Additional observations of neurobehavioral effects were reported (Levine et al., 1990; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981a; Levine et al., 1981b; Cholakis et al., 1980; Von Oettingen et al., 1949); however, a FOB conducted by Crouse et al. (2006) did not report any consistent, treatment-related behavioral effects. Further, Crouse et al. (2006) noted that the ability of the FOB to identify neurobehavioral effects at doses ≥8 mg/kg-day was limited due to the timing of the dosing procedure and timing of the FOB screenings. Given the reports of neurobehavioral effects in several studies, additional systematic evaluation of neurobehavioral effects would be informative. Hess-Ruth et al. (2007) reported possible transfer of RDX to offspring during gestation, as well as the presence of RDX in the milk of dams, indicating a potential for lactational transfer of RDX to offspring. Given the potential for exposure during gestation and lactation and the neurotoxic potential of RDX, the lack of a developmental neurotoxicity study was identified as a data gap. A $UF_D$ of 3 was applied to all PODs to account for limitations in neurobehavioral and neurodevelopmental testing.

Table 2-2 is a continuation of Table 2-1 and summarizes the application of UFs to each $POD_{HED}$ to derive a candidate value for each data set. The candidate values presented in the table below are preliminary to the derivation of the organ/system-specific reference values. These candidate values are considered individually in the selection of a representative oral reference value for a specific hazard and subsequent overall RfD for RDX.

**Table 2-2. Effects and corresponding derivation of candidate values**

| Endpoint and reference | $POD_{HED}$[a] | POD type | $UF_A$ | $UF_H$ | $UF_L$ | $UF_S$ | $UF_D$ | Composite UF | Candidate value (mg/kg-d) |
|---|---|---|---|---|---|---|---|---|---|
| Nervous system (rats) | | | | | | | | | |
| Convulsions Crouse et al. (2006) | 0.27 | $BMDL_{01}$ | 3 | 10 | 1 | 3 | 3 | 300 | $8.8 \times 10^{-4}$ |
| Convulsions Cholakis et al. (1980) | 0.06 | $BMDL_{01}$ | 3 | 10 | 1 | 3 | 3 | 300 | $2.0 \times 10^{-4}$ |
| Kidney/urogenital system (rats) | | | | | | | | | |
| Prostate suppurative inflammation Levine et al. (1983) | 0.23 | $BMDL_{10}$ | 3 | 10 | 1 | 1 | 3 | 100 | $2.3 \times 10^{-3}$ |
| Male reproductive system (mice) | | | | | | | | | |
| Testicular degeneration Lish et al. (1984) | 2.4 | $BMDL_{10}$ | 3 | 10 | 1 | 1 | 3 | 100 | $2.5 \times 10^{-2}$ |

[a]$POD_{HED}$ values based on data from the rat were derived using PBPK modeling; the HED POD based on data from the mouse was derived using $BW^{3/4}$ adjustment (see Section 2.1.3 and discussion of the PBPK models in Appendix C, Section C.2.5).

Figure 2-2 presents graphically the candidate values, UFs, and $POD_{HED}$s, with each bar corresponding to one data set described in Tables 2-1 and 2-2.

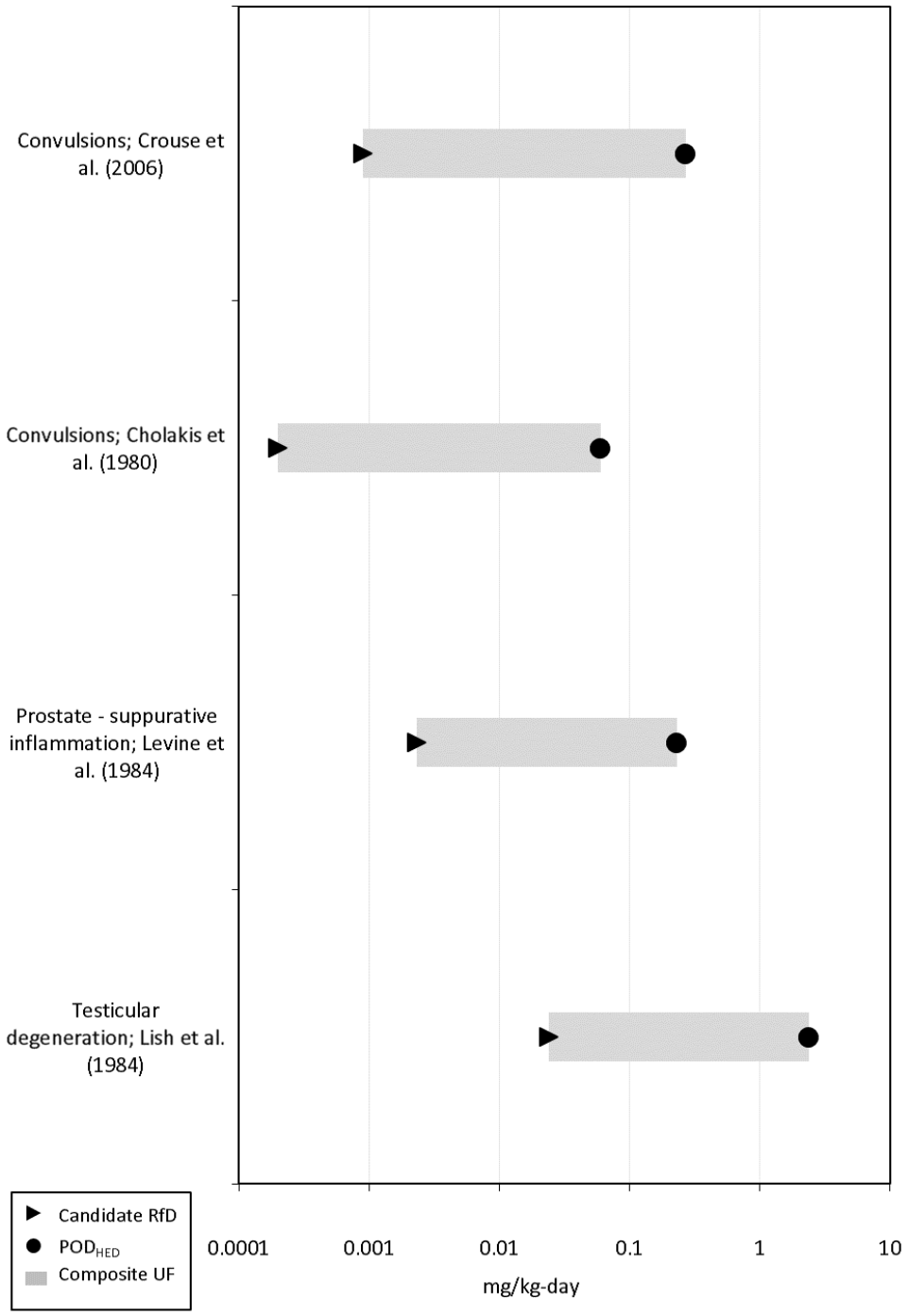



**Figure 2-2. Candidate values with corresponding POD and composite UF.**

### 2.1.4. Derivation of Organ/System-Specific Reference Doses

Table 2-3 distills the candidate values from Table 2-2 into a single value for each organ or system. 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 2-3. Organ/system-specific RfDs and proposed overall RfD for RDX**

| Effect | Basis | RfD (mg/kg-day) | Study exposure description | Confidence |
|---|---|---|---|---|
| Nervous system | Convulsions | $9 \times 10^{-4}$ | Subchronic | Medium |
| Kidney/urogenital system | Suppurative prostatitis | $2 \times 10^{-3}$ | Chronic | Low |
| Male reproductive system | Testicular degeneration | $2 \times 10^{-2}$ | Chronic | Low |
| **Proposed overall RfD** | **Nervous system** | **$9 \times 10^{-4}$** | **Subchronic** | **Medium** |

***Nervous System Effects***

The organ/system-specific RfD for nervous system effects was based on the incidence of convulsions in rats reported in Crouse et al. (2006), a well-conducted study that used a 99.99% pure form of RDX, five closely-spaced dose groups that provided a good characterization of the dose-response curve for convulsions, and an endpoint (convulsions) that was replicated across multiple other studies. Although the candidate value derived from Cholakis et al. (1980) is lower (by approximately fourfold), there is greater certainty in the value derived from Crouse et al. (2006) because of the longer exposure duration (90 versus 14 days), more systematic evaluation of neurobehavioral endpoints, and higher test compound purity.

***Kidney/Urogenital Effects***

A single data set for incidence of suppurative prostatitis in male B6C3F$_1$ mice as reported by Lish et al. (1984) was brought forward for quantitative analysis as a sensitive marker for the broader array of RDX-associated effects observed in the urogenital system. As previously discussed, the data supporting RDX-related kidney and other urogenital effects are largely limited to this 2-year study in the mouse. Accordingly, the candidate value for kidney and other urogenital effects is based on the incidence of suppurative prostatitis in male mice (Lish et al., 1984).

***Male Reproductive Effects***

A single dataset for male reproductive effects was brought forward for quantitative analysis: the incidence of testicular degeneration as reported in male B6C3F$_1$ mice exposed to RDX

in diet for 24 months (Lish et al., 1984). The candidate value for male reproductive effects is based on this dataset.

### 2.1.5. Selection of the Proposed Overall Reference Dose

Multiple organ/system-specific reference doses were derived for effects identified as potential hazards from RDX exposure, including nervous system effects, kidney and other urogenital effects, and male reproductive effects. Evidence for nervous system effects, and specifically convulsions, was observed in multiple studies, in multiple species, and following a range of exposure durations. In addition, the organ/system-specific RfD for nervous system effects was the lowest among the organ/system-specific RfDs derived for RDX. Evidence for dose-related effects on the urogenital system comes primarily from a single 2-year toxicity study in male rats (Levine et al., 1983), and evidence for male reproductive effects comes primarily from a single 2-year toxicity study in mice (Lish et al., 1984); neither a second chronic study in the rat that evaluated prostate histopathology nor a second mouse study was available to validate and replicate these findings.

The organ/system-specific RfD of **$9 \times 10^{-4}$ mg/kg-day** for nervous system effects in the rat as reported by Crouse et al. (2006) is selected as the overall RfD for RDX given the strength of evidence for the nervous system as a hazard of RDX exposure, and as the lowest organ/system-specific RfD. This overall RfD should provide an exposure level below which effects associated with RDX exposure are not expected to occur.

The overall RfD is derived to be protective of all types of effects for a given duration of exposure, and is intended to protect the population as a whole, including potentially susceptible subgroups (U.S. EPA, 2002). Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific lifestages of concern. Fluctuations in exposure levels that result in elevated exposures during these lifestages could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. In the case of RDX, no specific lifestages have been identified as a potentially susceptible subgroup.

### 2.1.6. Uncertainties in the Derivation of Reference Dose

The following discussion identifies uncertainties associated with the RfD for RDX. To derive the RfD, the UF approach (U.S. EPA, 2000a, 1994) was applied to a $POD_{HED}$ based on nervous system effects in rats exposed to RDX for a subchronic duration. UFs were applied to the $POD_{HED}$s to account for uncertainties in extrapolating from an animal bioassay to human exposure, the likely existence of a diverse population of varying susceptibilities, subchronic to chronic duration, and database deficiencies. These extrapolations are carried out with default approaches given the lack of data to inform individual steps.

Although the database is adequate for reference value derivation, uncertainty is associated with the consistency in toxicity results across studies that used RDX test materials that differed in

purity, formulation, and particle size. There is evidence that differences in test material formulation and particle size can affect absorption of RDX.

Nervous system effects have been documented in multiple studies and animal species and strains; however, there is some uncertainty associated with the incidence of reported neurological effects in studies that employed a study design that did not monitor animals with sufficient frequency to accurately record neurobehavioral effects, including convulsions.

### 2.1.7. Confidence Statement

A confidence level of high, medium, or low is assigned to the study used to derive the RfD, the overall database, and the RfD itself, as described in Section 4.3.9.2 of EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). The overall confidence in this RfD is medium. Confidence in the principal study (Crouse et al., 2006) is high. The study was well-conducted, utilized 99.99% pure RDX, and had five closely-spaced dose groups that allowed characterization of dose-response curves for convulsions. One limitation identified by study authors was the limited ability of the FOB to fully identify neurobehavioral effects at doses ≥8 mg/kg-day due to the timing of the dosing procedure and timing of the FOB screening. Confidence in the database is medium. The database includes three chronic studies in rats and mice; eight subchronic studies in rats, mice, dogs, and monkeys; two short-term studies; and four reproductive/developmental toxicity studies in rats and rabbits (including a two-generation reproductive study). Confidence is reduced largely because of limited examination of the potential for RDX to induce neurobehavioral and neurodevelopmental effects and the incomplete understanding of a MOA for convulsions. Reflecting high confidence in the principal study and medium confidence in the database, overall confidence in the RfD is medium.

### 2.1.8. Previous IRIS Assessment

The previous RfD for RDX, posted to the IRIS database in 1993, was based on a two-year rat feeding study by Levine et al. (1983). The no observed effect level (NOEL) of 0.3 mg/kg-day (LOAEL = 1.5 mg/kg-day) based on suppurative prostate inflammation in male F344 rats from this study was identified as the POD. An RfD of $3 \times 10^{-3}$ mg/kg-day was derived following application of an overall UF of 100 ($UF_A = 10$, $UF_H = 10$).

## 2.2. INHALATION REFERENCE CONCENTRATION FOR EFFECTS OTHER THAN CANCER

The RfC (expressed in units of $mg/m^3$) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or the 95% lower bound on the benchmark concentration (BMCL), with UFs generally applied to reflect limitations of the data used.

As noted in Section 2.1, human studies are generally preferred over animal studies as the basis for a reference value when quantitative measures of exposure are reported and the reported effects are determined to be associated with exposure. Of the available human epidemiological studies of RDX (West and Stafford, 1997; Ma and Li, 1992; Hathaway and Buck, 1977), none provided data that could be used for dose-response analysis. The studies by Ma and Li (1992) of neurobehavioral effects in Chinese workers and West and Stafford (1997) of hematological abnormalities in ordnance factory workers had numerous methodological limitations that preclude their use for quantitative analysis (see Literature Search Strategy | Study Selection and Evaluation). The study by Hathaway and Buck (1977) found no evidence of adverse health effects in munition plant workers, and therefore does not provide a basis for derivation of an RfC. Multiple case reports provide some evidence of effects in humans associated with acute exposure to RDX; however, while case reports can support the identification of hazards associated with RDX exposure, data from case reports are inadequate for dose-response analysis and subsequent derivation of a chronic reference value because of short exposure durations and incomplete or missing quantitative exposure information.

As discussed in the Literature Search Strategy | Study Selection and Evaluation, a single experimental animal study involving inhalation exposure was identified in the DTIC database; the study is not publicly available. However, the study would not have provided useful data on responses to inhaled RDX, as the study was limited by small numbers of animals tested, a lack of controls, and incomplete reporting of exposure levels. Therefore, the available health effects literature does not support the derivation of an RfC for RDX. Further, a PBPK model for inhaled RDX is not available to support route-to-route extrapolation from the RfD.

### 2.2.1. Previous IRIS Assessment

An RfC for RDX was not derived in the previous assessment posted to the IRIS database in 1990.

## 2.3. ORAL SLOPE FACTOR FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question, and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure.

### 2.3.1. Analysis of Carcinogenicity Data

As noted in Section 1.2.2, EPA concluded that there is "suggestive evidence of carcinogenic potential" for RDX. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) state:

> When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, 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.

In the case of RDX, the carcinogenicity of the chemical has been evaluated in one oral chronic/carcinogenicity bioassay in mice (Lish et al., 1984) and two bioassays in rats (Levine et al., 1983; Hart, 1976). The data in Lish et al. (1984) demonstrated a statistically significant positive trend with dose[6] in the incidence of liver and lung tumors in female, but not male, B6C3F$_1$ mice associated with dietary administration of RDX. In the study by Levine et al. (1983), the incidence of liver tumors in male F344 rats showed a statistically significant positive trend with dose[7]. No increases in tumors were observed in Sprague-Dawley rats exposed to RDX (Hart, 1976). As discussed further below, the 2-year studies by Lish et al. (1984) and Levine et al. (1983) were well-conducted studies that support quantitative analysis. Considering these data along with the uncertainty associated with the suggestive nature of the weight of evidence, EPA concluded that quantitative analysis of the tumor data may be useful for providing a sense of the magnitude of potential carcinogenic risk.

The incidences of liver and lung tumors in female mice from the study by Lish et al. (1984) were selected for quantitative dose-response analysis. The study by Lish et al. (1984) was performed in accordance with FDA Good Laboratory Practice regulations (FDA, 1979), included comprehensive histopathological examination of major organs, contained four dose groups and a control, used adequate numbers of animals per dose group (65/sex/group, plus interim sacrifice groups of 10/sex/group at 6 and 12 months) and a sufficient overall exposure duration (2 years), and adequately reported methods and results (including individual animal data). Female mouse liver tissues from the original unpublished study by Lish et al. (1984) were reevaluated by a pathology working group (PWG) (Parker et al., 2006) in order to apply more up-to-date histopathological criteria established by Harada et al. (1999). The updated liver tumor incidences from the PWG reanalysis of Lish et al. (1984) were used for quantitative dose-response analysis.

In the case of both liver and lung tumors, benign and malignant tumors (i.e., adenomas and carcinomas) were combined for dose-response analysis because benign and malignant tumors in both organs develop from the same cell line and there is evidence for progression from benign to the malignant stage (U.S. EPA, 2005a; McConnell et al., 1986).

Female mouse liver and lung tumor incidences from the Lish et al. (1984) study are summarized in Table 2-4.

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[6]A two-sided asymptotic Cochran-Armitage test yielded p = 0.041 for liver tumors and p = 0.019 for lung tumors in female mice.

[7]A two-sided exact Cochran-Armitage test yielded p = 0.032 for liver tumors in rat. An exact test was done because the incidence of tumors was too low for the asymptotic test to be reliable.

**Table 2-4. Incidence of hepatocellular and alveolar/bronchiolar tumors in female B6C3F$_1$ mice administered RDX for 2 years in diet**

| Tumor type | Study/Analysis | Dose group (mg/kg-day) | | | | |
|---|---|---|---|---|---|---|
| | | Control | 1.5 | 7 | 35 | 107[a] |
| Hepatocellular adenomas or carcinomas | Parker et al. (2006) | 1/67 | 4/62 | 5/63 | 10/64 | 4/31[b] |
| Alveolar/bronchiolar adenomas or carcinomas | Lish et al. (1984) | 7/65 | 3/62 | 8/64 | 12/64 | 7/31[b] |

[a]TWA dose, due to reductions in the highest dose from 175 to 100 mg/kg-day at week 11.
[b]Histopathology results are based on animals that survived more than 12 month. The smaller number of mice in the high-dose group reflects the high mortality at a dose of 175 mg/kg-day.

The incidence of liver carcinomas in male F344 rats from the study by Levine et al. (1983) was also considered for quantitative dose-response analysis. Although the study was well conducted (see Section 1.1.5), EPA considered that the association between RDX exposure and rat liver tumors is not strong, reflecting the relatively low magnitude of the rat liver carcinoma response and reduced confidence that the high-dose group accurately reflects lifetime cancer incidence because, in part, of low survival. A candidate slope factor is provided in Appendix D, Section D.2. for comparison.

### 2.3.2. Dose-Response Analysis—Adjustments and Extrapolations Methods

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) recommend that the method used to characterize and quantify cancer risk from a chemical be determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear approach is recommended when there are MOA data to indicate that the dose-response curve is expected to have a linear component below the POD or when the weight of evidence evaluation of all available data are insufficient to establish the MOA for a tumor site (U.S. EPA, 2005a). In the case of RDX, the mode of carcinogenic action for hepatocellular and alveolar/bronchiolar tumors is unknown. Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with RDX exposure.

The survival curves were compared across dose groups in each study to determine whether time of death should be incorporated in the dose-response analysis of tumors. For female mice in Lish et al. (1984), the survival curves were similar across dose groups after the dose was reduced in the high dose group to 100 mg/kg-day; therefore, a time-to-tumor analysis was not necessary for this study.

Tumor incidence was modeled using the multistage-cancer models in BMDS (versions 2.4 and 2.5). A standard BMR of 10% extra risk was applied to both tumor sites in the mouse.

Given the finding of an association between RDX exposure in the female mouse and increased tumor incidence at two tumor sites, basing the oral slope factor on only one tumor site could potentially underestimate the carcinogenic potential of RDX. Therefore, an analysis that combines the results from the mouse liver and lung tumor incidence is preferred. The MS-COMBO procedure (BMDS, version 2.5), extends the multistage-cancer models to the case with multiple tumors assuming independence between tumor types. There is no known biological relationship between liver and lung tumors in RDX-exposed mice, and therefore, as noted by the National Research Council (NRC, 1994), this assumption of independence is considered not likely to produce substantial error in risk estimates. MS-COMBO analyzes tumor incidence as present if either organ (or both) has a tumor and absent otherwise. The procedure derives a maximum likelihood estimate of the combined risk at a 95% confidence level based on the parameter values obtained for the individual tumor multistage model fits.

EPA's preferred approach for extrapolating results from animal studies to humans is toxicokinetic modeling. As described in Appendix C, PBPK models for RDX in mice and humans published by Sweeney et al. (2012b) were evaluated and further developed by EPA. Consideration was given to whether the available toxicokinetic information supported using an internal dose metric derived by PBPK modeling. The available mechanistic data (Section 1.1.5) point to some evidence, although not conclusive, that RDX-generated metabolites may be implicated in the observed tumorigenicity in the female mouse. However, there are no data on the toxicokinetics of RDX metabolites, and metabolism in the liver is the only route of elimination of RDX in the PBPK model. In this case, as is to be expected from mass balance principles, the PBPK modeling provides no further information; the HED obtained from the model-estimated amount of total RDX metabolites scaled by $BW^{3/4}$ was equal to that calculated using administered dose scaled by $BW^{3/4}$. In addition to the lack of data on metabolism, other major uncertainties were identified in the mouse PBPK modeling; EPA's evaluation of these uncertainties is summarized briefly in Section 2.1.3 and in more detail in Appendix C, Section C.2.5. Therefore, the PBPK model developed for the mouse was not used, and consistent with the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the preferred approach for calculating an HED from the mouse tumors is adjustment of the administered dose by allometric scaling to achieve toxicological equivalence across species.

As discussed in Section 2.1.1, the administered dose in animals is converted to an HED on the basis of $(\text{body weight})^{3/4}$ (U.S. EPA, 1992). This was accomplished by multiplying administered dose by $(\text{animal body weight in kg/human body weight in kg})^{1/4}$ (U.S. EPA, 1992), where the body weight for the mouse is 0.035 kg and the reference body weight for humans is 70 kg (U.S. EPA, 1988). It was not necessary to adjust the administered doses to HEDs prior to BMD modeling because the relationship between the two dose metrics is linear and the same POD would be produced whether the adjustment was performed before or after modeling. Details of the BMD modeling can be found in Appendix D, Section D.2.

### 2.3.3. Derivation of the Oral Slope Factor

The lifetime oral cancer slope factor for humans is defined as the slope of the line from the BMR (10% extra risk) at the BMDL to the estimated control response at zero (slope factor = $0.1/BMDL_{10\text{-HED}}$). This slope, a 95% upper confidence limit (UCL) on the true slope, represents a plausible upper bound on the true risk. The PODs estimated for each mouse tumor site are summarized in Table 2-5. Using linear extrapolation from the $BMDL_{10\text{-HED}}$, human equivalent oral slope factors (OSFs) were derived for each tumor site individually and both sites combined and are listed in Table 2-5.

**Table 2-5. Model predictions and oral slope factors for hepatocellular and alveolar/bronchiolar adenomas or carcinomas in female B6C3F$_1$ mice administered RDX in the diet for 2 years (Lish et al., 1984a)**

| Tumor type | Selected model | BMR | BMD (mg/kg-d) | BMDL (mg/kg-d) | POD = $BMDL_{10\text{-HED}}$[a] (mg/kg-d) | OSF[b] $(mg/kg\text{-}d)^{-1}$ |
|---|---|---|---|---|---|---|
| Hepatocellular adenomas or carcinomas[c] | Multistage 1° | 10% ER | 64.2 | 32.6 | 4.89 | 0.020 |
| Alveolar/bronchiolar adenomas or carcinomas | Multistage 1° | 10% ER | 52.8 | 27.7 | 4.16 | 0.024 |
| Liver + lung tumors | Multistage 1° (MS-COMBO) | 10% ER | 29.0 | 17.7 | 2.66 | 0.038 |

[a]$BMDL_{10\text{-HED}} = BMDL_{10} \times (BW_a^{1/4}/BW_h^{1/4})$, where $BW_a$ = 0.035 kg, and $BW_h$ = 70 kg.
[b]Slope factor = BMR/$BMDL_{10\text{-HED}}$, where BMR = 0.1 (10% extra risk).
[c]Incidence of female mouse liver tumors from Lish et al. (1984) are those reported in the PWG reevaluation (Parker et al., 2006).

An OSF was derived from the $BMDL_{10\text{-HED}}$ based on significantly increased incidence of hepatocellular and alveolar/bronchiolar adenomas or carcinomas in female B6C3F$_1$ mice (i.e., the Liver + Lung $BMDL_{10\text{-HED}}$ from MS-COMBO). The OSF of **$0.04\ (mg/kg\text{-}day)^{-1}$** is calculated by dividing the BMR (10% extra risk) by the Liver + Lung $BMDL_{10\text{-HED}}$ and represents an upper bound on cancer risk associated with a continuous lifetime exposure:

$$\text{OSF} = 0.1 \div (\text{Liver + Lung})\ BMDL_{10\text{-HED}}$$

$$= 3.8 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}$$

$$= 4 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}, \text{ rounded to one significant figure}$$

### 2.3.4. Uncertainties in the Derivation of the Oral Slope Factor

A number of uncertainties underlie the cancer unit risk for RDX. Table 2-6 summarizes the impact on the assessment of issues such as the use of models and extrapolation approaches (particularly those underlying the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a)), the effect of reasonable alternatives, the approach selected, and its justification.

**Table 2-6. Summary of uncertainty in the derivation of the cancer risk value for RDX**

| Consideration and impact on cancer risk value | Decision | Justification |
|---|---|---|
| Selection of study<br>The cancer bioassay in the rat (Levine et al., 1983) would provide a lower estimate of the OSF | Lish et al. (1984) as principal oral study to derive the human cancer risk estimate | Lish et al. (1984) was a well-conducted study; five dose levels (including control) used, with a sufficient number of animals per dose group (at terminal sacrifice, n = 62−65/dose group except highest dose where n = 31). Tumor data from the mouse provided a stronger basis for estimating the OSF than rat data, and yielded a higher (and therefore more health protective) estimate of risk than data from the rat bioassay. |
| Species/gender<br>Use of data sets from the male mouse would not support quantitative analysis of carcinogenic risk | OSF based on tumors in female mouse | It is assumed that a positive tumor response in animal cancer studies indicates the agent can have carcinogenic potential in humans in the absence of data indicating animal tumors are not relevant to humans (U.S. EPA, 2005a). As there are no data to inform whether the response in any given experimental animal species or gender would be most relevant for extrapolating to humans, tumor data from the most sensitive species and gender were selected as the basis of the OSF. |
| Combined tumor types<br>Human risk would ↓ if OSF based on analysis using only a single tumor type | OSF based on liver and lung tumors in female mouse | Basing the OSF on one tumor site could potentially underestimate the carcinogenic potential of RDX, so an analysis that included data from the two tumor sites was chosen to calculate the combined risk. Because there is no known biological dependence between the liver and lung tumors, independence between the two tumor sites was assumed. This is not likely to produce substantial error in the risk estimates (NRC, 1994). |
| Selection of dose metric<br>PBPK models are available for the rat, mouse and human, and using an appropriate internal metric can ↑ accuracy in human extrapolation. | Mouse liver and lung tumors: use administered dose | Lack of sufficient data on RDX metabolism and major uncertainties identified in the mouse PBPK model. |

| Consideration and impact on cancer risk value | Decision | Justification |
|---|---|---|
| Cross-species scaling<br>Alternatives could ↓ or ↑ slope factor (e.g., 3.5-fold ↓ [scaling by body weight] or ↑ 2-fold [scaling by $BW^{2/3}$]) | $BW^{3/4}$ scaling (default approach) | There are no data to support alternatives. Because the dose metric was not an area under the curve, $BW^{3/4}$ scaling was used to calculate equivalent cumulative exposures for estimating equivalent human risks. While the true human correspondence is unknown, this overall approach is expected neither to over- or underestimate human equivalent risks. |
| BMD model uncertainty<br>Alternative models could ↓ or ↑ slope factor | Use multistage model to derive a BMD and BMDL for combined tumor incidence | No biologically-based models for RDX are available, and there is no a priori basis for selecting a model other than the multistage. The multistage model has biological support and is the model most consistently used in EPA cancer assessments (Gehlhaus et al., 2011). |
| Low-dose extrapolation approach<br>↓ cancer risk would be expected with the application of nonlinear extrapolation | Linear extrapolation from the POD | Where the available information is insufficient to establish the MOA for tumors at a given site, linear extrapolation is recommended because this extrapolation approach is generally considered to be health-protective (U.S. EPA, 2005a). Because the MOA for RDX-induced liver and lung tumors has not been established, linear low-dose extrapolation was applied consistent with EPA guidance. |
| Statistical uncertainty at the POD<br>↓ OSF by 1.6-fold if BMD used as the POD rather than the BMDL | BMDL (default approach for calculating plausible upper bound slope factor) | Lower bound is 95% CI on administered exposure at 10% extra risk of liver and lung tumors. |
| Sensitive subpopulations<br>↑ OSF to an unknown extent | Considered qualitatively | No data are available to support a range of human variability/sensitivity in toxicokinetics or toxicodynamics for RDX, including whether children are more sensitive than other life stages. |

### 2.3.5. Previous IRIS Assessment: Oral Slope Factor

The previous cancer assessment for RDX was posted to the IRIS database in 1990. The oral slope factor in the previous cancer assessment was based on the bioassay by Lish et al. (1984) and analysis of data for hepatocellular adenomas or carcinomas in female mice. A slope factor of $1.1 \times 10^{-1}$ $(mg/kg\text{-}day)^{-1}$ was derived using a linearized multistage procedure (extra risk). This differs from the slope factor for hepatocellular tumors in Table 2-6, because the current OSF is based on the combined incidence of hepatocellular and alveolar/bronchiolar adenomas or carcinomas, PWG reevaluation of female mouse liver tumors, and use of scaling by body weight to

the 3/4 power for cross-species extrapolation (whereas the previous assessment scaled by body weight to the 2/3 power).

## 2.4. INHALATION UNIT RISK FOR CANCER

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the inhalation unit risk is a plausible upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

An inhalation unit risk value was not calculated because inhalation carcinogenicity data for RDX are not available. A PBPK model for inhaled RDX is not available to support route-to-route extrapolation from the OSF.

## 2.5. APPLICATION OF AGE-DEPENDENT ADJUSTMENT FACTORS

As discussed in the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), either default or chemical-specific age-dependent adjustment factors (ADAFs) are applied to account for early-life exposure to carcinogens that act through a mutagenic MOA. Because no chemical-specific data on life-stage susceptibility for RDX carcinogenicity are available, and because the MOA for RDX carcinogenicity is not known (see Section 1.1.5), ADAFs were not applied.

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