SUMMARY REPORT

OF THE EXTERNAL PEER REVIEW OF THE DRAFT

TOXICOLOGICAL PROFILE FOR

RDX

Submitted to:

The Agency for Toxic Substances and Disease Registry
Division of Toxicology
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QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by ATSDR. ATSDR confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled ATSDR's selection criteria. Reviewers conducted the review according to a charge prepared by ATSDR and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to ATSDR

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SECTION I

PEER REVIEWERS' SUMMARY COMMENTS

SUMMARY COMMENTS RECEIVED FROM

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COMMENTS ON ATSDR'S TOXICOLOGICAL PROFILE FOR RDX

The document under peer review is an update of an earlier version of the toxicological profile for RDX. Therefore, new information since the last version released in 1995 is reflected in this document. Generally speaking, it would be helpful if all important changes can be highlighted, and a separate chapter or section is dedicated to discuss all the new lines of evidence. Most importantly, it should be summarized and may be presented in the "Update Statement" whether there is any new evidence that has caused changes in the derived levels of significant exposure (LSE) or minimal risk level (MRL).

CHAPTER 1. PUBLIC HEALTH STATEMENT

This chapter has been written in a non-technical style suitable for the average citizen. No additional explanation is required for the scientific terms used. It also provides answers that adequately address the concerns of the lay public. These answers are consistent and supported by the technical discussion in the remainder of the text except for the following:

In section 1.9 on page 6, some regulations and recommendations for RDX are listed. An important error is that the level in workplace air stated here (1.5 mg/m^3) is not set by OSHA, but by NIOSH according to Table 8-1 "Regulations and Guidelines Applicable to RDX". An important omission is ACGIH's TLV (0.5 mg/m^3) listed in the same table, which is lower than NIOSH's REL.

OSHA = Occupational Safety and Health Administration; NIOSH = National Institute for Occupational Safety and Health; ACGIH = American Conference of Governmental Industrial Hygienists; TVL = threshold limit values; REL = recommended exposure limit.

Some typos in this chapter:

1. Page 2, Line 3 (table): Line 4 in the table "plants. RDX and can enter…" should read "plants. RDX can enter…".
2. Page 3, Line 2 (table): Line 2 in the table "RDX or breath in the dust…" should read "RDX or breathe in the dust…".

CHAPTER 2. RELEVANCE TO PUBLIC HEALTH

This chapter has evaluated the source and routes of RDX exposure, interpreted the significance of existing toxicity data related to human health, and derived MRLs. The documented human effects are limited to a few publications that reported human exposure incidents. Acute toxicity data from many animal studies

support the neurological effects, primarily seizures and convulsions, observed in humans. No human study on intermediate and chronic oral administration of RDX is available, thus data from animal studies were used to derive MRLs. It is appropriate and widely accepted to use animal (especially mammals like mouse, rat, pig, rabbit and dog) data in combination with an uncertainty factor for such purpose as deriving threshold values. Systemic and developmental toxicities of RDX observed in animals are relevant to human health because similar effects may occur in humans. Therefore, I agree with those effects known to occur in humans as reported in the text, and I think that the effects observed in animals are of great concerns to humans, and that exposure conditions (route, duration, or level) in both human and animal studies have been adequately described.

CHAPTER 3. HEALTH EFFECTS

Section 3.1 INTRODUCTION

Although this introduction is standard language, some brief substance-specific information may be added. For instance, no epidemiological investigations on RDX are available nor presented, but information related to RDX genotoxicity, mechanisms of action and biomarkers do exist. It would be appropriate not to mention “epidemiological investigations”, but desirable to mention briefly the RDX-specific sections that will follow in the text.

Section 3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

Toxicity - Quality of Human Studies

- Most of the reported human studies are isolated case studies where exposure to RDX occurred as a result of accidents via inhalation, oral ingestion or dermal contact. Some of the incidents causing acute toxicities ranging from systemic (e.g., gastrointestinal, cardiovascular and hematological) effects to neurological dysfunctions are often associated with unknown or unspecified doses. Others like a chronic inhalation exposure study (Hathawat and Buck, 1977) revealed no toxic effects in workers exposed to RDX dusts. These and other limitations of the human studies are sufficiently described in this section. Therefore, adequately designed human studies (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors) cannot be identified.
- The conclusions drawn by the authors of the cited human studies are appropriate and accurately reflected in the profile text.

- No NOAELs and/or LOAELs can be derived from the human studies due to the limitations mentioned above, which are provided in details in the text and adequately justified for excluding NOAELs/LOAELs.
- Although statistical tests are not described in the profile, the cited studies conform to sound statistical principles and have used, where necessary, scientifically appropriate statistical tests to evaluate collected data.
- I am not aware of any other studies that may be important and relevant in evaluating the toxicity of RDX.

Toxicity - Quality of Animal Studies

- Unlike human studies, there are plenty of adequately designed animal studies out there in the public domain, most of which have been identified in the text. For instance, the 90-day rat study conducted by the U.S. Army (U.S. Army 2006) was very well designed with adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels.
- The animal species used in the studies are appropriate for the most significant toxicological endpoint of the study.
- The conclusions drawn by the authors of the studies are appropriate and accurately reflected in the text.
- Wherever there exists sufficient data, appropriate NOAELs and LOAELs are derived for appropriate toxicological endpoints such as systemic, neurological and developmental effects.
- The profile text has also discussed the toxicities of RDX metabolites such as MNX (1-nitroso-3,5-dinitro-1,3,5-triazaine), DNX (1,3-dinitroso-5-nitro-1,3,5-triazaine) and TNX (1,3,5-trinitroso-1,3,5-triazine). For instance, it is described on page 43 lines 25~29 that two studies (Meyer et al. 2005; Smith et al. 2007) both show that RDX is more potent than its metabolites.
- To the best of my knowledge, appropriate statistical tests have been used in the data interpretation of the cited studies, and the statistical analysis of study data have been conducted properly.
- I am not aware of any other relevant studies being excluded from the profile.

Levels of Significant Exposure (LSE) Tables and Figures

- With the explanations provided in the "Users Guide", the LSE tables and figures are complete and self-explanatory. They present clearly and effectively information such as

accurate exposure levels (units, dose), the route of exposure, animal species, evaluated toxicological endpoints, and so on. No improvement is needed for the LSE tables and figures.

- I think it is INAPPROPRIATE to categorize toxicological effects into "less serious" and "serious" though this is in contrary to ATSDR's stand. Because effects are dose-dependent, the higher the dose is, the more severe the toxicity gets. I would suggest replacing the LOAEL "serious" with an LD50 or LD20. If serious effects occur, an LD50/20 can be derived. Otherwise, no LD50/20 is available.
- Two MRLs for the oral exposure route have been derived, one for the acute duration, and the other for the intermediate duration. The acute MRL was derived on the basis of a 14-day NOAEL of 8.5 mg/kg/day for tremors and convulsions (U.S. Army 2006). However, Figure 3-1 and Table 3-1 clearly show that two other studies reported lower NOAELs of 2 mg/kg/day (U.S. Army 1980b) and 6 mg/kg/day (U.S. Army 1986d) in Sprague-Dawley rats. It is unclear why the U.S. Army 2006 study was selected over the others in the MRL derivation. More explanation is required to justify the selection.
- The derivation of the intermediate MRL is justifiable but still needs some explanation on why the U.S. Army 2006 study was the sole study selected among all the available studies.
- No chronic MRL has been derived because, if derived from available data, it would be higher than the intermediate MRL.

Evaluation of Text

- The text has provided adequate descriptions on experimental conditions and designs for all cited studies. I find the Summary Table for Toxicity Studies for Exposure to RDX particularly helpful while reading the text because most of the information presented and discussed in the text is reflected in the table.
- The text has not evaluated the observed effects (or LOAEL/NOAELs derived from toxicity endpoints) that were reported in the studies for their relevance in both humans and animals. Such critical evaluation for relevance is not warranted here because all cited studies are obviously relevant to RDX effects on either human or animal health.
- No "bottom-line" statement can be located in the text which is made regarding the relevance of the endpoint for human health.
- Given the overall database, conclusions made in the profile are appropriate except for the acute oral MRL as discussed above.
- Adequate attention has been paid to dose-response relationships for animal data. Due to the lack of dose-response human data, no effort was made in this regard.

- The animal data has been used to draw support for any known human effects, where and when appropriate. Such practice is widely considered valid. For instance, neurological dysfunctions such as seizure and convulsion are consistently observed in both humans and animals as a result of acute exposure. Recovery often occurs within a few days or weeks as reported in both humans and animals. A high similarity exists in RDX effects between humans and animals, possibly due to similar modes of action.

Section 3.3 GENOTOXICITY

- No comments required.

Section 3.4 TOXICOKINETICS

- Absorption, distribution, metabolism, elimination and excretion of RDX in both humans and animals have been adequately discussed where data is available.
- No information has been identified regarding the distribution of RDX in human. Limited animal studies show no organs or tissues that RDX would preferentially accumulate in.
- All applicable metabolic parameters as well as all available pharmacokinetic/ pharmacodynamic models and supporting data have been presented, providing a complete coverage of the limited information for RDX.
- The scarcity of RDX toxicokinetics data in humans has made it impossible to even discuss the differences in toxicokinetics between humans and animals.
- The relevance of animal toxicokinetic information for humans has been discussed in adequate details.
- This section has presented both in vitro and in vivo information regarding RDX metabolism in animals. However, no study has been located with regard to the toxicokinetics of RDX metabolites. Therefore, a discussion of the toxicokinetics of different forms of RDX is inapplicable.

Section 3.5 MECHANISMS OF ACTION

This section has provided a brief overview of known pharmacokinetic and toxicological mechanisms, followed by a discussion of problems associated with animal to human extrapolations. It is my opinion that all possible mechanisms of action have been fully discussed.

Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

No information is available for RDX.

Section 3.7 CHILDREN'S SUSCEPTIBILITY

No comments required.

Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

- The identified biomarker of RDX exposure is the chemical itself as measured in blood or urine and hence specific for RDX.
- The measurements of blood or urine are valid tests to measure the biomarker of RDX exposure, which is consistent with statements made in other sections of the profile.
- No known specific biomarker of effect can be identified to characterize effects caused by RDX via inhalation, oral or dermal exposure.
- There are no valid tests to measure biomarker of effect. This is consistent with statements made in other sections of the text

Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

- This section has presented all but limited information on the interactive effects with other substances such as HMX and TNT. No information is available regarding the joint effects of RDX and other chemicals that might occur at hazardous waste sites
- The text has discussed the mechanisms involved in the antagonistic interaction between TNT and RDX.

Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

- The text states that there are no known populations that are unusually susceptible to RDX toxicity. Base on the state-of-the-art in this research area, I have to agree with this statement.

Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

- The first part of the section (3.11.1) has introduced generally recommended treatments found in the literature to reduce peak absorption of RDX following inhalation, oral, and dermal exposure. However, none of these treatments are specific for RDX. There is no controversy associated with the treatments, which are mostly standard practices like moving patients to fresh air after inhalation exposure, washing hands immediately after handling

RDX, and washing exposed eyes with eye drops. These treatments should not cause hazards to any specific populations because there is no population unusually susceptible to RDX.

- The second part of the section (3.11.2) has discussed methods to enhance the elimination of absorbed RDX. However, according to the text, no specific methods have been located in the literature that can effectively reduce body burdens of RDX. Meanwhile, hemodialysis, as reported by Kucukardali et al. (2003), was unable to reduce the RDX level in patients' serum three hours after ingestion.
- The last part of the section (3.11.3) has presented some standard treatments for convulsive activities and seizures that are often induced by RDX exposure. There is no information regarding whether such treatments can prevent RDX from reaching the target organs such as blood and brain. There is no known controversy associated with these treatments for RDX-intoxicated patients, nor is there any hazard known associated with any particular population because an unusually susceptible population has yet to be identified.

Section 3.12 ADEQUACY OF THE DATABASE

Existing Information on Health Effects of RDX

- To the best of my knowledge, this document has covered all existing studies related to health effects of RDX.

Identification of Data Needs

- The data needs are presented in a neutral, non-judgmental fashion without any bias.
- I do agree with most of the identified data needs except for the genotoxicity and mechanisms-oriented research. As stated in the text, existing genotoxicity data obtained from either microbial mutagenicity studies or human/mammalian studies have all showed negative results. It is most likely that RDX does not target nucleic acids (DNA or RNA). I can't see how epidemiological studies may help resolve this issue. Instead, research employing a mechanism-driven approach such as toxicogenomics or a combination of genetics, molecular biology and bioinformatics can probably uncover the molecular targets of RDX. On the other hand, the text did not identify toxicity mechanisms of RDX as an area where data is extremely scarce. In section 3.5 Mechanisms of Action, only five reports were identified (see 3.5.2 Mechanisms of Toxicity). It is now widely accepted that elucidation of modes of action can save researchers from looking at every possible target, which is both expensive and time-consuming.
- The text not only has indicated whether any information on the data need exist, but also adequately justified why further development of the data need is desirable.

Some typos found in this chapter are as follows:

Page 43, Line 17: "The metabolism of RDX has been studied in some detail miniature pigs…" should read "The metabolism of RDX has been studied in some detail in miniature pigs…".

Page 54, Line 24: "thus, an intermediate-duration inhalation MRL could be…" should read "thus, an intermediate-duration inhalation MRL could not be…".

Page 58 Line 5: Delete "or exposed to RDX dust".

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

- All the information and values presented in the chemical (Table 4-1) and physical properties (Table 4-2) tables are correct. I am not aware of anything missing in these two tables.
- As there is no other form known for RDX, information on various forms of the chemical does not exist.

CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

- Information provided in this chapter is accurate, up to date, and adequate. No relevant existing information is missing.

CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE

- The text has appropriately traced RDX from its point of release to the environment until it reaches the receptor population. The text has also provided sufficient and technically sound information regarding the extent of occurrence at NPL sites. To the best of my knowledge, I am not aware any other relevant information.
- The text has fully covered pertinent information relative to transport, partitioning, transformation, and degradation of RDX in all environmental matrixes including air, water, sediment, soil and biota (vegetation). There is no other relevant information missing from the text.
- The text has provided information on levels monitored or estimated in the environment. As RDX is a synthetic compound, it does not exist in the nature, and its background level is supposed to be zero. Proper units have been used to describe RDX for each medium. The information presented in this chapter includes the form of RDX measured and there is an adequate discussion of the quality of the information. No other relevant information exists as far as I know.
- The text has described both sources and pathways of exposure for the general population and occupations involved in the handling of RDX, children, as well as populations with

potentially high exposures. The selection of these populations is appropriate. No additional populations should be included in this section.

- Identification of Data Needs (Sections 6.8.1) is presented in a neutral, non-judgmental fashion without any bias. I completely agree with the identified areas of data needs. Adequate justification has been provided as to why further development of the data need would be desirable.

One typo has been identified in this chapter:
Page 79 Line 13: Delete "in" at the end of this line.

CHAPTER 7. ANALYTICAL METHODS

- Tables 7-1 and 7-2 have summarized analytical methods for determining RDX in biological and environmental samples, respectively. The coverage is thorough and complete. I am unaware of additional methods that can be added to the tables.
- It is unclear whether these methods can be used to measure RDX metabolites. No methods specifically for measuring key metabolites (e.g., MNX, DNX and TNX) have been included.
- The text has provided necessary discussions on the pros and cons of most analytical methods with regard to their sensitivity, precision, scalability, and so on. When unique issues related to sampling for the substance exist, they have been adequately addressed in the text. For instance, some methods such as the HRGC/ECD (high-resolution gas chromatography/ electron capture detector) require sample clean-up (Douse 1982), while others like HPLC/PMDE (high-performance liquid chromatography/pendant mercury drop electrode, Lloyd 1983) and HPLC or HRGC/TEA (thermal energy analyzer, Fine et al. 1984) do not.
- Identification of Data Needs (Sections 7.3.1) is presented in a neutral, non-judgmental fashion without any bias. I completely agree with the identified areas of data needs. Adequate justification has been provided as to why further development of the data need would be desirable.

CHAPTER 8. REGULATIONS AND ADVISORIES

- I am unaware of other regulations or guidelines that may be appropriate for the table.

CHAPTER 9. REFERENCES

- To the best of my knowledge, there are no additional references that provide new data or better studies than those already in the text.

UNPUBLISHED STUDIES (IF APPLICABLE TO REVIEW)

- Four studies have been classified as "unpublished" in the text and the References, three of which have been cited in the text.

  (1) A study listed as "U.S. Army 2007a" deals with physiologically based pharmacokinetic (PBPK) modeling of RDX in rats. The content of this study has actually been published in a peer-reviewed journal – Journal of Applied Toxicology, which is listed as Krishnan et al. 2009 in the References. Therefore, this study is excluded from my assessment.

  (2) A study listed as "U.S. Army 2007b" is cited once as "unpublished" in the text (see Page 37 Lines 15~16) but listed without labeling such in the References. A double check on the document included on the CD reveals that this study is indeed "unpublished" and has not gone through any peer-review process (both internal and external). The following should be added at the end of this entry: [Unpublished study to be peer reviewed].

  (3) A study listed as "U.S. Army 2008" is labeled as "unpublished" in the References. However, the main part of the study has been published as Bannon et al. (2009) in a peer-reviewed journal Chemical Research in Toxicology.

  (4) An unpublished study by Major M. (2005) has been listed only in the References without any citation in the text or the supplementary documents. Therefore, an evaluation of this study is not applicable and should not be required.

- I have reviewed two of the unpublished and cited documents (U.S. Army 2007b and U.S. Army 2008). My assessment of both documents is as follows: Both studies were adequately designed, conducted and reported. Experimental protocols and QA/QC procedures were closely followed. The collected data were analyzed using appropriate statistical methods. The reported results are valid and of high-quality, and authors' conclusions are scientifically sound. There is no inadequacy or confounding factors that may alter the conclusions.

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PEER REVIEW

TOXICOLOGICAL PROFILE FOR RDX

General:

There are some issues that relate to adults and children that were not indicated in the profile that might be considered. It was presumed in the profile that the discussion of exposure that individuals may only be exposed by a single route be it inhalation, dermal or orally. However, combination of more than one route of exposure was not mentioned.

Chapter 1. Public Health Statement

This chapter is well-written and presents information in a manner that the lay public can readily understand the issues surrounding the production of RDX, generation of fumes, uses, consequences of exposure arising from contact with RDX and the basis for using these chemicals. In general, the queries in the major headings adequately address the questions posed to allay the concerns of the lay public in an informed "scientific" manner. The summary statements are discussed in depth subsequently in the profile (Chapters 2-8) and are consistent with the statements in Chapter 1. The following are issues to be considered for Chapter 1.

1) Page 1 line 17 states that exposure to RDX involves inhalation, ingestion, imbibition or dermal routes. However these are not the sole routes of exposure for humans. There are also indirect routes, for example 1) in the pregnant state the fetus or unborn child can be exposed to RDX via the mother and 2) the suckling or lactating infant can be exposed indirectly through breast milk. These 2 indirect routes need to be included in this paragraph as the text on 4 describes these routes. Further exposure to more than one route is also a possibility and needs to be stated.

2) Page 1 line 21 change sex to more appropriate term "gender"

3) Page 2 line 3 in box " Removal from soil.."' It states that "It does not build up in fish…" change to " RDX does not bioconcentrate or bioaccumulate in fish…" (this is the case with mercury). This page is submitted identifying change.

4) Page 3 line 5 under box for Laboratory animals. It states that "RDX for 3 months or longer had

decreased body weights and slight liver and kidney damage" What is meant by "slight" Is this transient? Does this disappear? Is this effect important? Is it reversible? This page is submitted identifying change.

5) Page 1 line 22 in addition to the factors mentioned such as age, family traits, lifestyle and state of health, other factors that need to be included are physiological status, for example pregnancy. Secondly kidney function as the chemical is eliminated in urine and does not accumulate. In the presence of abnormal renal function adverse effects may be manifested due to higher amounts of the chemical. Thus, inclusion of function of kidneys involved in elimination of these chemicals is important. It is well-established that in neonates the function of kidney is not fully developed and thus this subpopulation may be at higher risk. Although this has not been reported for humans, this needs to be stated with respect to making the public aware.

6) Page 4 line 1 box for Cancer. Change to "There are no studies of cancer reported in people exposed to RDX". This page is submitted identifying change.

7) Page 4 line 6 box Laboratory animals. It states that " …RDX during gestation gave birth to slightly smaller babies.." Was this in length? Was this in body weight? Was this permanent? Was this critical in later life? This page is submitted identifying change.

8) Page 4 line 6 box Breast milk Change to "….RDX who nurse their babies could transfer RDX to the …" this page is submitted identifying change.

Chapter 2.Relevance to Public Health

This is a generalized section divided into 3 subsections. The Section 2.1 provides an adequate well-written background describing the environmental sources, uses, presence of RDX in the biosphere .The most likely exposure routes for occupational humans being inhalation of vapors and particulates and dermal contact. Exposure to the general population may be attributed to water and food; soil for children; and/or inhalation of vapors or particulates as well as dermal contact be it soil or occupational. However, it should be stated that exposure may also occur as a result of multiple routes e.g. in drinking water and by dermal contact or by dermal and inhalation occupationally. Section 2.2 is a concise, comprehensive summary of the health effects and this is followed by section 2.3 describing the MRL (Minimal Risk Levels). This chapter covers the subject matter in an introductory fashion which is subsequently followed by a Chapter that reports the same material in far greater depth. As a background section, all the key

factors, tissue health effects and derivation of MRL are adequately covered. The inability to derive an inhalation MRL using animal and human data is clearly stated. The derivation of oral MRL is appropriate for the acute and intermediate-duration exposures based on the available scientific data with neurological effects as the most sensitive biomarker are appropriate. Due to an insufficient database it was not possible to derive an oral chronic MRL for RDX. The conclusions reached at the end of each subsection are appropriate based on the fact that all the key studies were incorporated into the profile. The exposure conditions have been adequately described. The following are specific points to address:

9) Page 8 line 16 the term " very" has no biological meaning. Hence delete or state actual % solubility.

10) Page 9 lines 2-3 exposure can also occur by " …dermal contact with soil containing RDX and/or by inhaling.." that is by more than 1 route

11) Page 9 lines 8-11 state that exposure occupationally can occur by inhalation and dermal (i.e, by more than 1 route)

12) Page 9 line 15 the term "very" has no biological relevance. Hence delete this term.

13) Page 10 line 6 the term "slightly" is not biologically relevant. Hence delete as the actual quantitative value is provided.

14) Page 10 line 14 the term « Small » has no biological meaning. Is this significant ? then state ?

15) Page 10 line15 what does "minor" mean? Is this significant?

16) Page 10 line 17 what does a "slight impairment" mean? Was this significant? Was this transient?

17) Page 10 line 18 insert "..decreases in serum alanine …"

18) Page 10 line 27 change nonsignificant to " ..quantitative increase…" .

19) Page 10 line 29 insert "Adverse developmental effects…"

20) Page 11 line 1 insert "No adverse developmental…"

21) Page 12 line 30 delete "slightly" as the actual numerical value is provided.

22) Page 13 line 7 insert "…found no significant systemic effects.."

23) Page 13 line 10; page 14 line 28 delete "slightly" as actual numerical value is provided.

24) Page 13 line 21 change high arousal to "Increased arousal.."

25) Page 14 line 16 term "Small" has no biological meaning. Is this significant?

26) Page 15 line 3 one can NOT increase mortality. Change to "..increases in incidence of mortality.."

27) Page 15 line 14 insert "..decrease in serum cholesterol…"

28) Page 16 line 1 change condition to "..a common manifestation.."

Chapter 3.Health Effects

Quality of Human Studies

The human studies reported were not experimentally designed studies. Occupational exposures via inhalation or dermal routes are known; however, the sample sizes are far too small to calculate statistical relevance. Human oral and inhalation exposure data due to accidental ingestion of RDX are available but the exposure concentrations are not known and the sample size is exceedingly small. Adverse neurological manifestations were the critical effects clearly identified as the predominant target site for RDX following oral or inhalation exposure. This was clearly stated in the profile and no specific conclusions were drawn from the human case studies (sample size was exceedingly small) except to record manifestations, symptoms and mortality which are appropriate as confounding factors, lifestyle, etc. were not controlled. The human data from oral, inhalation and dermal routes of exposure to RDX were not used to derive a NOAEL or LOAEL as again there was an absence of relevant data derived from these studies. Statistical evaluation of the human inhalation, oral and dermal exposure data was not undertaken which is appropriate and justifiable in light of the paucity of data.

29) On page 17 lines 17 in the description of health effects, it states "...and dermal) and then by health effect (death, systemic...". Death is NOT a health effect but an ultimate consequence. The text needs to be changed to reflect this.

30) On page 18 lines 34 insert "..reported adverse gastrointestinal, hematological…"

Quality of Animal Studies

In general, the animal studies were carried out under the guidelines of Animal Care Committee approval under Good Laboratory Practice (GLP) regulations at predominantly at governmental (US Army or US Navy) facilities. The animal studies described refer to the oral route as inhalation exposure of rabbits and guinea pigs resulted in pneumonia and pulmonary congestion with unspecified RDX levels. The oral animal RDX studies were variable with respect to design, number of animals and strain. It is clearly stated in the document where the limitations exist with respect to presence and/or absence of statistical analysis of data and hence relevance of biological findings. The use of only one dose and one strain also limited the biological relevance and utility of some findings reported. There were a sufficient number of dose-levels considered and gender was taken into account in some studies. As the studies were carried out under control conditions in lab settings, variables other than RDX were accounted for and thus if mortality occurred the predominant cause was this chemical. The control groups were treated appropriately to adequately compare to RDX and thus it was possible to attribute a cause- effect relationship to the chemical. The animal species used and types of various tests for organ specificity were appropriate and in agreement with guidelines from the EPA or NTP. The conclusions reached as well as the statement of the limitations of biological relevance of data were appropriately addressed. The appropriate NOAEL and LOAEL were identified in the relevant studies. The parent RDX was used to generate data and the metabolites, where known were always identified. Data was subjected to the appropriate analysis and used correctly to identify biological relevance. The following are specific points to address:

31) On page 21 lines 25, & 26 change cause to more appropriate term "produced" as chemicals produce but do NOT cause the effect.

32) Page 21 lines 27 & 28 you can NOT have an excessive death. Death is the final outcome. Change to " …an excessive incidence or number of deaths"

33) Page 22 lines 6& 21 insert " ...no significant changes…"

34) Page 22 line 24 define what is meant biologically as " slight myocardial…"Is this significant?

35) Page 23 line 9 change "..produce histopathological alterations…"

36) Page 23 lines 22, 24 & 32 "..significant hematological effects ……"

37) Page 23 line 26 & 30; page 24 line 6, 12 & 30 define biologically what is meant by "..slight". Is this significant?

38) Page 24 line 1; page 25 line 1& 13 insert "No histopathologic changes were ...."

35) Page 24 lines 12 & 19 define clearly the term "minor.."

39) Page 25 lines 18 & 21; page 26 line 11 insert "..No significant ...."

40) Page 26 line 6 insert "…adverse immunologic effects…"

41) Page 26 line 9 change to " ..failed to reveal any marked pathological alterations.."

42) Page 27 line14 insert "..no marked neurological effects.."

43) Page 29 line 3 change nonstatistically significant to "..quantitative decrease.."

44) Page 29 line 12 insert "..adverse developmental.."

45) Page 29 line 24-25 insert " no adverse fetal.."

46) Page 30 line 27 delete term "small" as you havec provided the actual values

47) Page 31 line 3 insert " regarding adverse respiratory.."

48) Page 31 lines 13 & 16 insert " No adverse effects.."

49) Page 31 line 25 "…no significant changes…"

50) Page 31 lines 31 change to "..no pathological alterations.."

51) Page 32 line 12 define what is meant by "slight". Is this relevant?

52) Page 32 line 31 what is meant by "small, transient" Is this biologically relevant?

53) Page 33 line 18 change to "..to induce DNA…

54) Page 37 lines 3 & 5 "kidneys"

55) Page 43 lines 22& 26; page 45 line 6 change caused or causing to "..produced or producing.."

56) Page 44 line 15 " possess potent anticholinesterase…."

57) Page 44 line15-16 if it is "small but significant" then it is simply "significant". There are no degrees of significance. Delete the small and but.

58) Page 44 line 23 change small nonsignificant to simply "numerical decrease.."

59) Page 45 line 7 what is meant by "transient"

60) Page 45 line 20 state specifically what is a "subconvulsive dose"

61) Page 45 line 27 state specifically what is a :high" dose or amount

62) Page 28 lines 9 & 32 insert " No significant histological .. "

63) Page 28 line 17 "..regarding adverse reproductive.."

64) Page 28 line 19 " no pathological changes.."

65) Page 28 lines 23 & 31delete nonstatistically significant and change to "quantitative.."

66) Page 56 line 26 change a nonsignificant to "..quantitative increase..'

67) Page 56 line 32 insert " No significant changes.."

68) Page 57 line 15 define what is meant by "mild.."

LSE Tables and Figures

Table 3-1 and Figure 3-1 in Appendix B in are understandable but there are points that need clarification. There is an indication for: c, cat; n, mink; e, gerbil; etc. in the Figure 3-1 legend but these species are not presented. The legend should only specify the species for which data are provided or available. The categorization of effects as "serious" or "less serious" is clearly defined in the text. The limitations to this categorization are recognized and stated in the text. The values obtained to derive the MRL in Appendix A from acute and intermediate duration oral administration studies in animals were appropriate for the

specific compounds evaluated. An oral MRL was derived for RDX where data were available while data resulted in no derivation of a chronic oral MRL as the NOAEL and uncertainty factor of 100 would be higher than the intermediate duration MRL and this is appropriate.

Evaluation of Text

The major limitations of the studies for inhalation, oral, and dermal exposure were stated within each of the described subsections. For example under Systemic Effects it states the limitations and subsequently within the effects such as Gastrointestinal Effects, Hematological Effects, Hepatic, Respiratory Effects, etc the limitations are again reiterated, especially the paucity of data for some organ systems. The relevance of data generated has been clearly defined as to the species studied including human as well as animal. The endpoints for human health are clearly stated at the start of each subsection and the conclusions drawn are justifiable based on the overall database. Dose-response data were not available for human studies as in most cases exposure were accidental or occupational and not under controlled lab settings. The dose-response data from animal studies was limited in value in establishing the inhalation MRL. The dose-response data generated for RDX oral MRL was appropriately used to calculate either an acute or intermediate duration MRL. Where applicable the reliable NOAEL and LOAEL were reported. The human data arose from accidental or occupational exposure to RDX to unknown amounts and durations whereas the animal studies were dose controlled. Reports in human exposure report predominantly significant adverse neurological effects and similarly, in animals exposure to excessive amounts of RDX also predominantly affected the central nervous system. Hence the use of changes in central nervous system functions is justifiable as a biomarker of exposure for humans.

Toxicokinetics

The absorption, distribution, metabolism, and excretion of RDX following inhalation, oral or dermal exposure is adequately addressed. The major tissues for RDX, where data are available, to which accumulation or lack of accumulation occurs have been identified. Although data for the most part are not available for humans the presumption in this section is that all organs are functioning normally. However, the kidney is a key tissue involved in elimination of RDX. Although highly unlikely considering that the concentration to which a human is exposed is far less than in animal, it is possible that in conditions of subjects with renal dysfunction, the chemical may accumulate in the body. Whether the level reached is sufficient to produce an effect is dependent on the quantity of chemical exposure, which could be high in an industrial accident and exaggerate the neurological manifestations. The animal studies were controlled for toxicokinetic parameters using predominantly the oral route by exposing animals to RDX and data are available for absorption, distribution, metabolism and excretion; but with human exposure obtaining information on distribution and metabolism is not feasible and exposure to only one chemical is not

always possible be it occupational or ingestion.. This latter fact is noted in "real-life" human exposure where drinking water contains a multitude of chemicals. The tissue distribution and metabolic profile for RDX was adequately stated in the text and the model for PBPK is adequately presented in the text. Little is known regarding human data with respect to toxicokinetics for RDX. Animal data are available with respect to target organs but comparisons with respect to toxicokinetics to humans are clearly not justifiable. There is adequate discussion of toxicokinetics for RDX where studies are available.

Mechanisms of Action

This section adequately describes the known mechanisms of absorption, distribution, metabolism and excretion with respect to RDX. All known mechanisms of action have been stated.

Biomarkers of Exposure and Effect

The measurement of human blood, urine or feces may serve as a biomarker for exposure to RDX but is of limited value as this may be a metabolite or parent compound. It also does not distinguish between acute or chronic exposure. The use of seizures as a biomarker for RDX is too non-specific as numerous other agents produce this effect. There are no specific biomarkers of effect identified for RDX in humans.

Interactions with Other Substances

There is an adequate discussion on the interactive effects between other substances and RDX. Data are provided on the interactive effects of RDX and TNT and the manifestations seen but there are no mechanistic descriptions. The paucity of data on mechanisms whereby interactions occur are adequately described. Hazardous waste sites contain various chemicals but as the mechanism of action of RDX remains unknown one can not speculate on chemical interactions.

Populations that are Unusually Susceptible

It is clear from the text that there are no known specific populations that are at increased risk for RDX adverse health effects in humans and this is comprehensively covered.

Methods for Reducing Toxic Effects

The management is a generalized annotation for procedures to follow for exposure to compounds and involves basically avoidance in the case of dermal washing exposed skin for dermal (with soap and water) ocular by irrigation of eyes with water and for oral exposure (using activated charcoal). There are no specific antedotes for RDX but emesis should not be used in case of oral poisonings. In the case of convulsions after RDX has reached the target tissue brain, administration of anticonvulsive therapy is recommended. There is no associated controversy associated with supportive therapy and this treatment is

applicable to adults and children. There are no treatments available specifically for RDX to reach a target tissue as washing the skin or eyes, or reducing absorption by using activated charcoal is common to all class of substances. This type of treatment is not controversial and is applicable for all age groups. The compounds are eliminated rather quickly and do not appear to accumulate in tissues.

Adequacy of the Database

The data needs are presented in a neutral non-biased fashion. The data needs as identified in the text are clearly stated. The text clearly identifies which studies are necessary to fill in knowledge gaps and how data generated would aid in establishing biomarkers of exposure and consequences of exposure on human health.

Chemical and Physical Information

Information is provided on RDX in tabular form. Data provided is adequate and complete.

Production, Import/Export, Use, and Disposal

Information provided is adequate.

Potential for Human Exposure

The text adequately describes the fate of RDX from release following industry-related to ammunition production, formulation, assembly, etc. into environmental media including air, water and soil. The text provides sufficient information regarding the occurrence and detection of RDX in air, water or soil at NPL sites. Pertinent information on transport, partitioning, transformation and degradation are covered clearly and stated concisely. The text provides adequate information on the levels of RDX monitoring in air, water, soil, sediment as well as other media such as agricultural crops and ocean floor fauna. The RDX levels are given in the appropriate units for each medium and occupational exposure levels are provided. The quality of data is adequately addressed. The text adequately addressed the sources and pathways for exposure in the general population and specifically to a potentially high- risk group such as infants and occupational exposed workers. A particular relevant subpopulation which could be included are individuals with kidney diseases, although studies to date have not focused on this population. The following are specific comments to address:

69) Page 67 line 31 delete term “slightly” which has no biological meaning…state actual %

70) Page 68 line 26; page 70 line 23; page 76 line 27 define what is meant by “very” as it has no biological meaning; hence delete term

71) Page 70 line 14 change to "..that produce photolytic.."

72) Page 72 line 3 change to "..concentration fell to 2.9 ppm…",

73) Page 75 line 16 "identified"

74) Page 75 line 24 insert "..no apparent RDX.."

75) Page 77 line 22-23& 29-30 it should state that exposure can occur via more than 1 route at the same time

76) Page 78 line 6 change assure to "ensure"

Analytical Methods

The analytical methodology for RDX has been addressed adequately. The following are points to address

77) Page 84 line 19; page 85 line 4 delete term "very" as accuracy or recovery has been defined

78) Page 85 line 14 change "slightly" to "numerically less"

79) Page 86 line 17 delete term good as recovery value provided

80) Page 87 line 34 change assure to "ensure"

Regulations and Advisories

I am not aware of other regulations or guidelines. The following point needs to be addressed:

81) Page 90 line 2 insert "..potential adverse health.."

References

There are no additional references to add to the text.

SUMMARY COMMENTS RECEIVED FROM

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COMMENTS ATSDR'S TOXICOLOGICAL PROFILE ON RDX

Child health and development:

Are there data relevant to child health and developmental effects of RDX?

I am unaware of any data in addition to the Woody et al (1986) case report and the experimental data from the deer mice study of Smith et al (2007) and U.S. Army reports on rodent developmental toxicity (1980b, 1986d). All have been well summarized in the profile.

Are there general issues relevant to child health not discussed?

The general issue of age-dependent development of the blood-brain barrier is the most pertinent to the critical toxicity of RDX. This is sufficiently discussed and appropriate resource material cited. Other relevant developmental changes generally affecting disposition of RDX are also adequately discussed and documented.

Public Health Statement:

The tone is informative at a level appropriate for the target audience. Questions posed outline the majors issues of concern to the public, namely how one would be exposed and health effects upon exposure. The emphasis on neurotoxicity of persons living near RDX-contaminated sites is the appropriate emphasis.

Chapter 2. Relevance to Public Health:

Are effects known to occur in humans reported?

I think a brief mention should be made of the hematological effects of transient anemia and methemoglobinemia shortly after acute exposure reported in case studies (Kucukardali et al 2003; Knepshield and Stone 1972) as a less serious effect of RDX. This effect would be predicted if denitration is a significant RDX metabolic pathway (Major and Reddy, 2007). Inconsistent results in replicating this effect with rats may be due to the greater levels of methemoglobin reductase in rat erythrocytes compared to those of humans (Smith, JE and Beutler, E [1966] Methemoglobin formation and reduction in man and various animal species. Am. J. Physiol. 210:347-350.). I would suggest one brief summary sentence at the end of the first paragraph of section 2.2, p. 8 and a comment added to section 2.3, p 13, line 1.

A minor issue is misspelling of identified on line 28, page 8. Also, insertion of "erythrocyte" before mean cell volume in line 13 of p. 15 would better define this parameter.

Chapter 3. Health Effects:

Section 3.2 Discussion of Health Effects by Route of Exposure

Were adequately designed studies identified and appropriate conclusions drawn and described? Were dose-response thresholds accurately identified?

I believe a comment suggesting that the hematological responses to oral RDX of the several species documented should include a comment that differences may reflect different levels of erythrocyte methemoglobin reductase as documented by Smith and Beutler (above) and Rockwood et al 2003 (attached). I've suggested addition of such a comment to p 23, line 26.

All other health effects in animals have been sufficiently documented and classified with respect to seriousness. Appropriate threshold values are derived.

Available human data have been comprehensively reviewed and limitations in study design and interpretation explicitly stated.

Appropriate studies have been included in the LSE tables and figures. Derivation of a safety level, such as done for the MRLs, using a 100-fold reduction from experimental dose preceding the lowest exhibiting the most sensitive adverse effect is a well accepted traditional practice. Usage of the LSE table and figure is clearly described in the Appendix B guide.

Section 3.3 Genotoxicity

Studies addressing genotoxicity for the parent compound RDX with a variety of endpoints are comprehensively reviewed and an overview of concordance among studies is well summarized in Table 3-3.

Section 3.4 Toxicokinetics

A clarification is suggested for the in vitro metabolism studies described in section 3.4.3. "Liver" should precede "microsomes" on p. 38, line 18.

For the discussion of the PBPK model validation in section 3.4.5, the discussion on application to the child should include a citation to the U.S. Army 2007 document. I suggest that citation should appear on p 42, line 1. Also, I am unable to locate simulations on high-low dose extrapolations for mice referred to on p 42 in either of the key supporting documents (U.S. Army 2007, Krishnan et al 2009) for the PBPK model validation.

Section 3.5 Mechanisms of Action

Mechanisms with sufficient support for the critical effect of neurotoxicity are described and references documented.

With respect to cytochrome P450 metabolism of RDX in miniature swine, the CYP2B4 notation

refers to the isozyme specific to rabbit. I am unable to correlate with a porcine ortholog, so have suggested modification of wording on p. 43, line18. A suggested modification of the description of the Meyer et al 2005 study has been added on p 43, line 27 to clarify study design.

A correction is needed on p. 44, line 12. Nerve "acts" should be corrected to nerve "gasses".

Section 3.6 Toxicities Mediated through the Neuroendocrine Axis

It is unclear to me why only in vitro studies were considered for inclusion in this section. I think the earlier cited reproductive studies, especially that describing testicular degeneration (U.S. Army 1983a), would have relevance here.

Section 3.7 Children's Susceptibility.

The key document, the Woody et al (1986) case report is adequately described. Supporting evidence from animal studies on deer mice (Smith et al (2007)) and rats (U.S. Army 1980b, 1986d) are presented. All have been well summarized in the profile.

General age-dependent aspects of relevant physiology are presented and supported with appropriate citations.

Section 3.8 Biomarkers of Exposure and Effect

The discussion of biomarker of exposure is limited to parent compound. Lack of information is appropriately invoked as the reason that metabolite determination has not been used as a biomarker of exposure. The lack of specificity of known responses to RDX is correctly discussed as a limitation of determination of biomarkers of effect.

Section 3.9 Interactions with Other Chemicals

Other munitions chemicals and formulation chemicals for RDX-based explosives that are the most likely co-contaminants of priority sites are listed. What information is available on their interactive effects is discussed.

Section 3.10 Populations that are Unusually Susceptible.

Correct statement of lack information on this topic. Speculation based upon emerging mechanisms is premature at this time for this survey document.

3.11 Methods for Reducing Toxic Effects

Routine emergency medicine procedures for reducing symptoms of chemical poisoning are described and are appropriate. Clinical experience with lack of success with hemodialysis is discussed. Available chemical specific information on skin decontamination is presented.

3.12 Adequacy of Database

A correction to section 3.12.2 Identification of Data Needs is needed on p 54, line 24.

This section highlights a need for additional chronic-duration studies to clarify a mechanism for previously observed prostatitis in rat. Although the effect has been attributed to a microbial infection, these studies should address RDX effects on host resistance that may have contributed to susceptibility of infection. I've added a comment to p. 55, line 23 to indicate this.

Since N-nitroso products of RDX have been shown to be minor metabolites (Major and Reddy 2007) and since the N-nitroso is a toxicophore often yielding genotoxicity, additional study of the genotoxicity of RDX metabolites is warranted. Identification of the genotoxic S9 metabolite of the tri-nitroso metabolite TNX is especially pressing (Pan et al 2007; George et al 2001) and determination of whether that S9 metabolite is produced by human liver microsomes is an important goal.

Indicated data needs are well justified and their application to a further understanding of the health effects of RDX is made clear. The reader can sense a prioritization through phrases such as "it would be useful" [to have additional studies] compared to "additional studies are necessary".

Chapter 4. Chemical and Physical Information

Tabulated values agree with source material.

Chapter 5. Production, Import/Export, Use and Disposal

I have no further knowledge of information relevant to this section.

Chapter 6. Potential for Human Exposure.

6.1 Overview

The abbreviation for hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) has been previously defined (p. 34). To be consistent with the use of abbreviations for DNX and TNX, MNX should be used.

Consistent units should be used for water values on p. 74 rather than switching between ppm and ug/L.

6.7 Populations with Potentially High Exposures.

Another potential population that may be exposed to high levels of RDX is that employed in demilitarization of nuclear, biological and chemical weapons as per international treaty agreements. Disassembly of these missiles involves disassembly of RDX-containing bursters and detonators.

Chapter 7. Analytical Methods

Listed methods are consistent with those for which I am knowledgeable. Detection limitations are specified and confounders associated with preparation of specific sample types acknowledged.

Chapter 8. Regulations and Advisories.

Table 8-1 indicates a superscript "i" associated with item b. water, EPA, National primary drinking water standards. It is unclear from the table footnotes to what this superscript refers.

Chapter 9. References

There are 2 references cited in the textual material that have been omitted from the reference list. These are:

Beller, HR. and Tiemeier, K (2002) Use of Liquid Chromatography/Tandem Mass Spectrometry to Detect Distinctive Indicators of In Situ RDX Transformation in Contaminated Groundwater. Environ. Sci. Technol. 36: 2060-2066.

And a citation to:
Pennington and Brannon 2002

Also, citation U.S. Army 1980d is out of order.

SECTION II

ADDITIONAL REFERENCES AND DATA SUBMITTED BY THE PEER REVIEWERS

ADDITIONAL REFERENCES AND DATA SUBMITTED BY

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Species Comparison of Methemoglobin Reductase

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Methemoglobin (MHb) formation is effective in treating cyanide (CN) poisoning. Endogenous activity of the enzyme MHb reductase (MR) reflects the capacity to reduce MHb and thus represents a key factor for evaluating anti-CN efficacy of MHb formers. MR activity was measured in whole blood of nine animal species and was compared with human MR activity. The animals in this comparative study included seven nonhuman primate (NHP) species, the beagle dog, and the ferret. Although exhibiting higher MR activity than in humans, the rhesus and aotus NHPs' average MR activity was the closest to humans', with raw data from each NHP showing overlap with human raw data. The beagle dog, used extensively to study anti-CN characteristics of MHb formers, was the sole species that displayed MR activity lower than in humans, with no data overlap. Based on MR activity, the rhesus and aotus NHPs may each represent a more accurate model for predicting human responses to MHb formers. The data from this study provides a unique interspecies enzyme comparison, which should facilitate future rational development of anti-CN MHb formers. Exp Biol Med 228:79–83, 2003

Key words: methemoglobin reductase; species comparison; nonhuman primates; cyanide; animal models

Cyanide (CN) is a primary nitrile present in all living animals and it also occurs in abundance in many plants such as sorghum, cherry, almond, bamboo, and cassava (1). CN is inexpensive and easy to manufacture from readily available components, and is accessible for a wide array of commercial activities. For example, CN is used in various industrial applications, such as electroplating, mining, and in the production of many synthetic fiber materials such as nylon (1). However, because it shows rapid, profound toxicity at adequate concentrations, CN has also been employed as a chemical threat agent. Although CN may not be an efficient large-scale offensive persistent chemical weapon, it has been used in military and terrorist operations (2), and it remains a recognized chemical warfare threat (3, 4). Historical use of CN as a military or terrorist weapon has been reviewed elsewhere (1, 5).

Due to CN's toxic nature and rapid onset of action, treatment must be administered as soon as possible after poisoning. Clinically, a prophylactic could be developed and administered prior to exposure (1). Many compounds that form methemoglobin (MHb) effectively counter CN toxicity (6–14). Mechanistically, CN has a stronger affinity for MHb than for cytochrome oxidase (15), the putative molecular receptor for CN toxicity (1), to form cyanomethemoglobin. Because of CN's toxicity profile, a long-acting MHb former has been recommended, potentially for use as a pretreatment (6, 16, 17). However, MHb itself cannot transport oxygen, and careful monitoring of MHb levels minimizes harmful side effects that can occur when MHb levels rise above about 20% of the total hemoglobin. These side effects include dyspnea, exercise intolerance, headache, fatigue (~20%–50% MHb), tachypnea, seizures, central nervous system depression and coma (~50%–70% MHb), and ultimately death (>70% MHb).

An MHb former for use as an anti-CN pretreatment is under consideration. In the 1940s, some cautioned against using an MHb former as a CN pretreatment in a military setting because of the potential reduction in the overall physical performance efficiency of military personnel pretreated with an MHb former (18). Nevertheless, newer MHb formers are now available, and research evaluating this approach continues. As part of this effort, the most appropriate and practical available nonhuman model must be identified. Model selection criteria for studies evaluating MHb formers include physiological and genetic enzyme profile similarities with humans (19).

Endogenous activity of the enzyme MHb reductase (MR) reflects an organism's capacity to reduce MHb and is therefore an important factor in evaluating the anti-CN efficacy of MHb formers. Under normal conditions, this NADH-dependent enzyme (also referred to as NADH MR,

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ferricyanide reductase, NADH diaphorase, or cytochrome b_5 reductase) is the only system within the erythrocyte that maintains hemoglobin in its oxygen-carrying reduced state. Toxicologically, MR is the rate-limiting enzyme controlling the toxicokinetics of the reduction of MHb, thus directly affecting the anti-CN efficacy of MHb formers. Species with lower MR activities convert MHb back to hemoglobin slower than do species with higher activities (20). It is noteworthy that a second form of this enzyme, which is NADPH dependent, is less active in mammals in converting MHb back to hemoglobin than is the NADH-dependent form (20). Deficiencies in the reductase systems and resulting clinical manifestations have been described elsewhere (21).

MR has been measured in humans, as well as in a variety of nonhuman species, including cattle, cat, dog, fruit bat, goat, guinea pig, horse, mouse, Northern brown bandicoot, platypus, rabbit, rat, red kangaroo, sheep, short-beaked echidna, wallaby, and wombat (22–24). Although studies evaluating MHb formers have been conducted in nonhuman primates (NHPs) (25–27), MR activity in NHPs has not been systematically evaluated (28, 29). Lacking specific data, Marrs *et al.* (30) speculated that MR activity in NHPs would likely resemble that observed in man, rather than the higher activity in species such as rodents.

This study evaluated normal MR activity in various NHP subspecies, and compared their MR activity with normal human MR activity. For additional comparison, MR was also evaluated in two other species, the ferret, and the beagle dog, the latter of which is often used in MHb studies (30). Presumably, the species with MR activity that most closely resembles that of man would provide the closest model to man for development of an effective, safe MHb class of CN pretreatments or improved antidotes and their active metabolites (31, 32).

Material and Methods

Whole blood samples from humans (Mayo Medical Laboratories, Rochester, MN), NHPs (United States Army Medical Research Institute of Chemical Defense [USAMRICD], the Southwest Regional Primate Research Center, and the New England Regional Primate Research Center), ferrets (Armed Forces Radiobiological Research Institute [AFRRI]), and beagle dogs (the Walter Reed Army Institute of Research [WRAIR] and AFRRI) were collected in acid citrate-dextrose (ACD) tubes (Table I). All samples were shipped cold (not frozen) overnight to the Mayo Medical Laboratories where normal, endogenous MR activities were determined using a standard enzyme-catalyzed reaction as depicted in Figure 1. Under these conditions, the samples are stable for 2 days at ambient temperature and for more than 20 days at 4°C. The standard Mayo Medical Laboratory MR assay was performed at 30°C for 12 min in the presence of Tris-HCl buffer, 2 m*M* NADH with or without 2 m*M* $K_3Fe(CN)_6$, and a 1:20 hemolysate mix as described previously (33–35). NADH oxidation was analyzed spectrophotometrically at 340 nm.

Preliminary *t* tests were performed as necessary to assess whether gender (i.e., beagle dogs, chimpanzees, and African Green NHPs only), anesthesia condition (AFRRI beagle dogs only), and/or laboratory (i.e., beagle dogs from WRAIR or AFRRI) were a significant source of variability. An analysis of variance (ANOVA) was subsequently performed on all species and a Dunnett's test was used to compare the human MR data against that of the other spe-

Table I. Test Groups for Measurement of Endogenous MR Activity

	n	M/F	Anesthestic(s)
Human data set			
Human *(Homo sapiens)*	30	NA	NA
Nonhuman primate data set			
African Green *(Chlorocebus aethiops)*	19	15/4	Telazol (3.0 mg/kg)
Aotus *(Aotus sp.)*	12	11/1	Ketamine (25 mg)
Baboon *(Papio anubis)*	10	10/0	Ketamine (10.0 mg/kg)
Chimpanzee *(Pan troglodytes)*	6	3/3	Telazol (10.0 mg/kg)
Cynomolgus *(Macaca fasicularis)*	6	6/0	Acepromazine (0.7 mg/kg) Ketamine (7.0 mg/kg)
Marmoset *(Callithrix jacchus jacchus)*	6	6/0	Ketamine (0.2 ml)
Rhesus *(Macaca mulatta)*	15	15/0	Telazol (3.0 mg/kg)
Other nonhuman species data set			
Ferret *(Mustela putorius furo)*	5	5/0	Ketamine (0.25 mg/kg) Xylazine (2.0 mg/kg)
Beagle *(Canis familaris, beagle)* (WRAIR)	15	12/3	No anesthesia
Beagle *(Canis familaris, beagle)* (AFRRI)[a]	7	0/7	No anesthesia
Beagle *(Canis familaris, beagle)* (AFRRI)[a]	7	0/7	Acepromazine (0.2 mg/kg) Diazepam (0.025 mg/kg) Ketamine (5.0 mg/kg) Isoflurane (1.5–2.0%)

[a] This single group of beagle dogs was sampled both under anesthesia as well as with no anesthesia in order to ascertain whether anesthesia affected MR activity. M, male; F, female; NA, not available.

$$2\ K_3Fe(CN)_6 + NADH \xrightarrow[\text{reductase}]{\text{ferricyanide}} 2\ K_2Fe(CN)_6 + NAD^+ + H^+$$

Figure 1. Principle of the MR assay conducted at Mayo Medical Laboratories (Rochester, MN). The activity at 30°C was followed by spectrophotometric analysis by measuring oxidation of NADH at 340 nm.

cies. For all analyses, statistical significance was maintained at $P < 0.05$.

Results

MR activity was quantified as International Units per gram of hemoglobin (IU/g Hb) following Mayo Medical Laboratories' standard procedures, and as used by others (36). See Beutler (33) for a more detailed description of the use of this unit of measurement. Analyses (*t* tests) indicated no significant effect of gender on MR activity. Therefore, MR activity data sets for the WRAIR beagles, chimpanzees, and African green NHPs were collapsed across gender for subsequent analyses. In addition, paired *t* tests conducted on the MR activity in beagle dogs sampled with or without anesthesia revealed no significant differences. Therefore, for subsequent analyses on these data, anesthesia condition was not included as a variable, and values for groups were averaged. A *t* test indicated that the MR activity in the beagle dogs from WRAIR was significantly higher than that observed in the beagle dogs from AFRRI. Therefore, these two groups of beagle dogs were subsequently evaluated as unique data sets.

The species differed statistically from each other (one-way ANOVA). Dunnett's tests to compare each species with humans indicated that all species were significantly different from humans. However, MR data as presented in a Box-and-Whisker plot (Fig. 2) (Box-and-Whisker plots are summary data plots based on the median, quartiles, and extreme values of a data set. The box represents the interquartile range [25th–75th percentiles] that contains 50% of the values. The whiskers are lines that extend from the box to the largest and smallest observed values that are less than 1.5 box lengths from either end of the box. The line across the box indicated the median of the data set. Outliers are 1.5–3 box lengths from the end of the box. Extremes are more than 3 box lengths from the end of the box.) illustrate that rhesus and aotus NHPs were quite similar to humans, with a great deal of overlap. Using the 25th and 75th percentiles, the beagle dog, the chimpanzee, the baboon, and the ferret all displayed higher MR activity, and failed to overlap with human MR activity (Fig. 2). However, MR data illustrate that rhesus and aotus NHPs were quite similar to humans, with a great deal of overlap. From raw data, the beagle dog was the only species tested that did not overlap with humans.

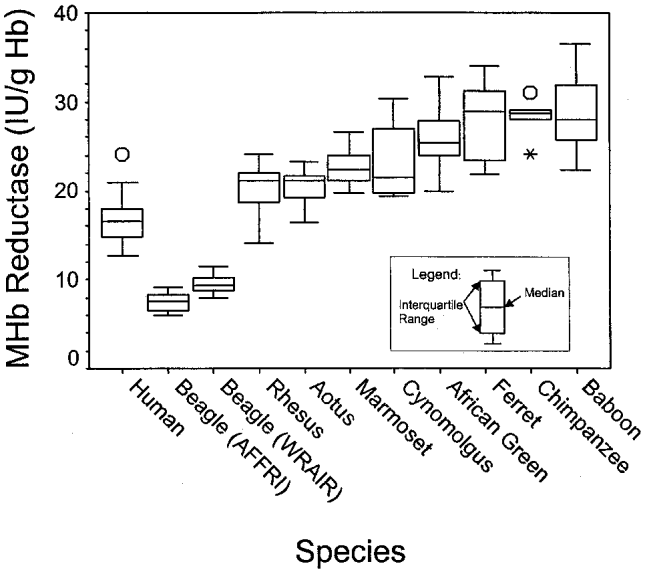

Figure 2. Box-and-Whisker plot of MR data. O and an asterisk represent outlier and extreme scores, respectively.

Discussion

In the present cross-species evaluation of endogenous MR, humans, seven NHP subspecies and two additional comparative nonhuman species were studied. When combined with previous reports (20, 22, 23), these results expand the database of known MR activity among nonhumans, and provide information vital for the selection and successful application of a nonhuman model for anti-CN MHb former research. Among the NHPs, comparison of MR activity revealed differences across subspecies. The rhesus and aotus NHPs exhibited MR activity closest to humans. These data support the continued use of the rhesus NHP in MHb former research (12). The aotus NHP may also be an alternative nonhuman model. The lower MR activity in the beagle dog relative to MR activity in humans, however, suggests caution when using this species as a nonhuman model in this particular area of research.

It is noteworthy that Srivastava and colleagues measured erythrocyte MR activity in a variety of nonhuman species, including the rhesus NHP and the beagle dog (29, 37). In one study (29), beagle dog MR activity was higher than the other species evaluated (rhesus NHP, rat, and mastomys). However, in a more recent study (37), beagle dog erythrocyte MR activity was reported to be significantly lower than MR activity in the rat, mastomys, mouse, and hamster. Furthermore, the beagle dog was described as being relatively MR deficient (37). Although the nature of differing MR results between these two studies (i.e., Refs. 29, 37) remain unclear, our data, showing low MR activity in the beagle dog, would generally support the results of the second study by Srivastava *et al.* (37).

The presence or absence of anesthetic agents was not addressed in previous MR species comparison studies (22, 23). It was determined in the present study that anesthesia did not significantly affect MR activity in the beagle dog. However, this limited evaluation should not preclude a more thorough study of this variable.

The authors recognize that the MR assay used in this study, although widely employed and accepted, uses an artificial substrate and does not measure MR directly. MR activity using this convenient artificial substrate is known to differ in magnitude from that determined using direct measurement; however, relative changes across experimental conditions within each methodology are similar (34).

In conclusion, a careful consideration of MR activity would be most prudent when selecting a nonhuman model for MHb studies. Data support the comparability of both the rhesus and aotus NHPs to humans, as MR activities were most similar. Relatedly, Huser (38) suggested that for hematologic studies, the rhesus NHP is the most preferable NHP model for man. Finally, the rhesus NHP data indicate that from a comparative point of view, this species more accurately resembles the human with respect to metabolic disposition (31).

The authors greatly appreciate the cooperation of Dr. James Hoyer from the Mayo Medical Laboratories (Rochester, MN) for conducting the MR assays, and for providing the human MR data set. WRAIR (Washington, DC), AFRRI (Bethesda, MD), the Southwest Regional Primate Research Center (San Antonio, TX), and the New England Regional Primate Research Center (Southborough, MA) kindly provided nonhuman blood samples. The authors thank Ms. Robyn B. Lee for her excellent statistical analysis of the data. Special recognition is extended to Ms. Anita Moran for expert technical assistance.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense.

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Methemoglobin formation and reduction in man and various animal species[1]

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SMITH, JOSEPH E., AND ERNEST BEUTLER. *Methemoglobin formation and reduction in man and various animal species.* Am. J. Physiol. 210(2): 347–350. 1966.—The rate of methemoglobin formation and reduction in man, goats, sheep, horse, cattle, and pigs was studied. Methemoglobin formation in the various species was studied by the addition of sodium nitrite to hemoglobin solutions, and methemoglobin reduction was carried out on nitrited washed erythrocytes using glucose and glucose plus methylene blue. It appears that the susceptibility to methemoglobin formation is related to the methemoglobin reduction rate in such a way that a rapid methemoglobin formation rate is offset by a rapid methemoglobin reduction rate.

comparative hematology; erythrocytes; methylene blue; ruminants; nonruminants

WITHIN RECENT YEARS the similarities and dissimilarities of proteins which serve the same function in different animal species have attracted much attention. This functional heterogeneity represents in most cases an adaptation to environmental needs of the animal's living conditions or metabolism. One classical example is the displacement to the left of the dissociation curve for fetal blood as compared to that of the maternal organism (1). Another such adaptation is the displacement of the characteristic oxygen dissociation curve to the right with diminishing size of the animal, which seems to be associated with the metabolic need for oxygen and is related to the unloading of oxygen in the tissues (14). This facilitates the unloading of oxygen at a relatively high tissue tension.

It has been reported that oxyhemoglobin oxidation to methemoglobin by sodium nitrite occurs at a rate which is characteristically different for each animal species, and that there is very little difference between individuals of the same species (3). Similarly, the reduction of methemoglobin has been shown to have considerable species variation (12). It is the purpose of this communication to point out a relationship between the susceptibility of oxyhemoglobin to oxidation and the rate of methemoglobin reduction in the erythrocytes of man and the larger domestic animals.

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MATERIALS AND METHODS

All blood samples were collected from mature individuals of the various species into ACD (acid-citrate-dextrose) solution to which sodium chloride had been added to the extent of 0.25% to increase the osmotic strength to a level more nearly isotonic with RBC's. The blood samples were stored at 4 C for up to 7 days prior to use. The rate of oxidation of oxyhemoglobin to methemoglobin was determined by the method of Betke et al. (3). Erythrocytes from the various species were washed three times with 1.5% sodium chloride solution and hemolyzed by dilution with distilled water. The hemolysate was mixed with 4–5 vol colloidal aluminum hydroxide and filtered. The hemoglobin concentration was determined by the cyanmethemoglobin method, adjusted to 105 mg/100 ml, and $\frac{1}{20}$ vol of 2.8 M phosphate buffer, pH 6.8, was added. The reaction was started by the addition of .02 ml freshly prepared .073 M sodium nitrite solution to 2.5 ml hemoglobin solution. The reaction rate was followed at 630 mμ in a Gilford model 2000 multiple absorbance recorder.

Methemoglobin reduction was carried out in the system previously described except that the volume of all reactants was reduced by one-third (4). Blood from the various species was centrifuged, and the plasma and buffy coat removed. The hemoglobin was converted to methemoglobin by incubating the erythrocytes with 2 vol .145 M sodium nitrite solution for 20 min. The cells were then separated by centrifugation and washed with 7–10 vol isotonic saline. The reaction mixture contained: nitrite-washed erythrocytes, 29%; potassium phosphate buffer, pH 7.4, 65 mM; glucose, 28 mM; and sodium chloride, 45 mM. Methylene blue, 1.7×10^{-2} mM, was used to activate the triphosphopyridine nucleotide-linked system. Methemoglobin percentage was determined by the method of Evelyn and Malloy (8) at 2-hr intervals for a total of 6 hr.

RESULTS

Methemoglobin formation. The formation of methemoglobin from a partially purified hemoglobin solution for

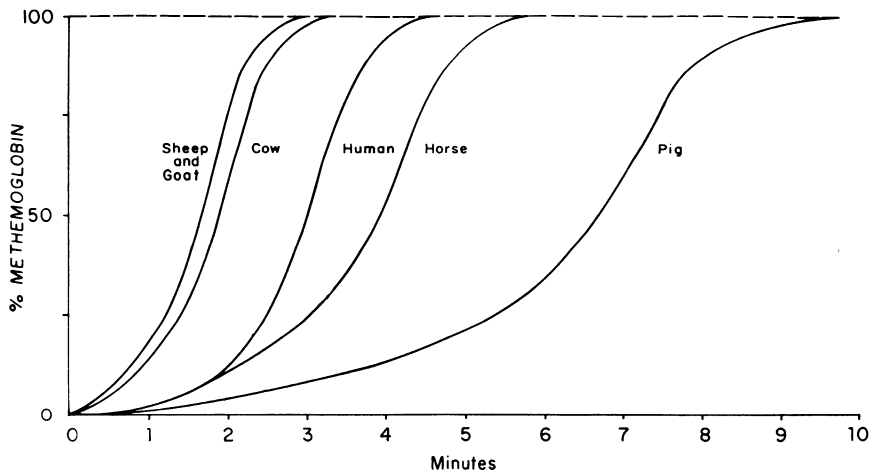

FIG. 1. Rate of formation of methemoglobin from partially purified hemoglobin of various animal species. Zero time was the time when .02 ml freshly prepared .073 M sodium nitrite was added to 2.5 ml hemoglobin solution.

the various species is shown in Fig. 1. It will be noted that the oxidation rate is specific for each species studied and occurs most rapidly in those animals of the suborder Ruminantia (sheep, goat, and cow) and is slower in nonruminants (man, horse, and pig). The oxidation in sheep and goats was similar and therefore, one line was drawn to represent both species.

Methemoglobin reduction with glucose alone. When nitrite-treated, washed erythrocytes were incubated with glucose, the reduction of methemoglobin was linear for the first 6 hr of incubation. As shown in Fig. 2, there is a marked difference between each of the species studied. The variation within a given species was usually small, and the results seen in this figure represent the average of two or more animals of the same species. With one exception (man), the animals with the most rapid reduction rate are ruminants.

Methemoglobin reduction with glucose and methylene blue. The rates of methemoglobin reduction with methylene blue and glucose (Fig. 2) again show marked differences between the various species. By comparing the results obtained with glucose with those obtained with glucose plus methylene blue (Fig. 2), the degree of acceleration which is produced in the presence of methylene blue is seen. This acceleration is most dramatic in man and cattle where the reduction rate is increased by over six- and fourfold, respectively. The change in sheep and goats is also quite high in terms of the absolute increase; however, since the rate of reduction without methylene blue in these animals is quite high, the relative increase is just under threefold. On the other hand, the increase seen in both the horse and pig is less (1.8 and 2.4), despite the fact that the reduction in absence of the dye is also low.

DISCUSSION

Of various domestic and laboratory animals, ruminants are most frequently reported to suffer nitrate poisoning. It is thought (11) that this is the case because nitrite is an intermediate in the reduction of nitrate to ammonia by the rumen microorganisms; however, in view of our results, at least a part of the increased sensitivity to methemoglobinemia by ruminants might also be due to the inherent differences in the susceptibility of the hemoglobin molecule to oxidation. Both the results of methemoglobin formation studies reported by Betke et al. (3) and those of the present report show that hemoglobin from ruminant erythrocytes is more easily oxidized to methemoglobin than the hemoglobin of nonruminants. Similarly, Bartels et al. (2), using potassium ferricyanide as the oxidizing agent, have shown that the hemoglobin of some ruminants (camel, yak, deer, and llama) were more easily oxidized than those of the nonruminants (man and elephant) studied.

Since methemoglobin reduction using glucose as substrate, which probably more nearly represents the in vivo conditions, occurs rapidly in ruminants, it would appear that the increased susceptibility to methemoglobinemia in these animals is offset by an increased ability to reduce methemoglobin. It would be reasonable to expect that the methemoglobin-reducing ability would be increased in those species in which the hemoglobin molecule is more susceptible to oxidation. In order to test whether the formation and reduction of methemoglobin were related and whether methemoglobin reduction was decreased in animals whose hemoglobin was more resistant to methemoglobin formation, the time when one-half of the hemoglobin had converted to methemoglobin was plotted in minutes as a function of methemoglobin reduction per hour, using glucose as a substrate (Fig. 3). The correlation coefficient computed from these results was $-.89$, which is statistically significant at the .05 level (15). It would thus appear that, although there is a large variation in the rate of formation of methemoglobin and its subsequent reduction, these two physiological processes are related.

Recently several investigators (6, 10, 13) have used a methemoglobin reduction test technique (5), which incorporates sodium nitrite, glucose, and methylene blue, to evaluate the pentose phosphate metabolism of various species. This test was developed for the detection of decreased shunt metabolism as an inherited condition in man and its use is dependent on a constant rate of methemoglobin formation. It is now apparent that this

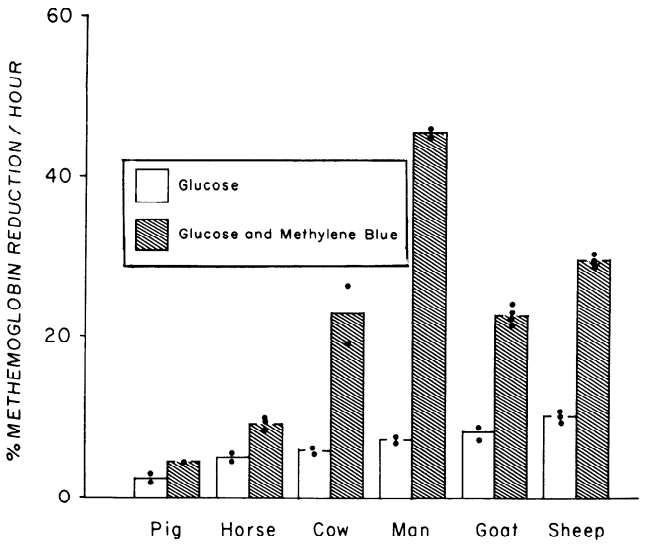

FIG. 2. Methemoglobin reduction in nitrite-washed erythrocytes of various animal species. Glucose concentration was 28 mM and methylene blue (when used) was 1.7×10^{-2} mM.

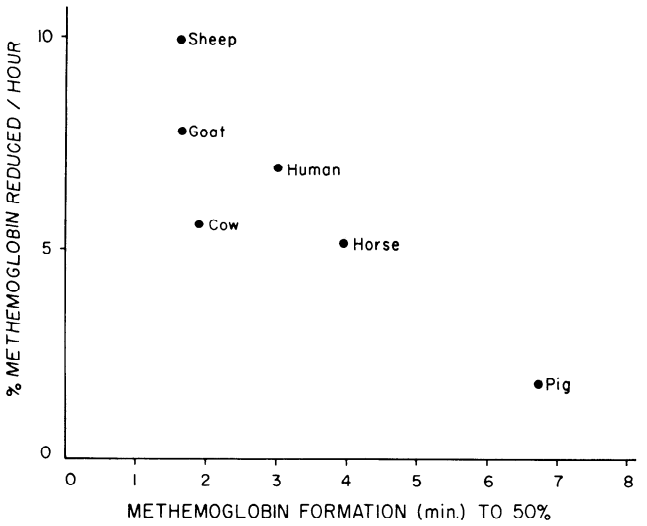

FIG. 3. Correlation of methemoglobin formation rate and methemoglobin reduction rate among various animal species. The methemoglobin formation rate was taken as the time when one-half of the hemoglobin had been converted to methemoglobin and the methemoglobin reduction rate was the methemoglobin reduced per hour using glucose as a substrate.

technique cannot be used indiscriminately in other species because of the variation in methemoglobin formation. For example, sheep and pigs are reported to have approximately the same amounts of methemoglobin remaining at the end of the 3-hr incubation period (13), yet the results of the experiments by Matthies (12) and those reported in this study show that these species vary greatly in their ability to reduce methemoglobin in a methylene blue-linked system.

The lack of stimulation of methemoglobin reduction by methylene blue in swine and equine erythrocytes may be due either to a relative deficiency of TPNH oxidase or the ineffectiveness of the dye as a substrate for the enzyme. Such a lack of linkage has been reported in monkeys (9), and apparently was the cause of the failure of Budtz-Olsen et al. to find glucose 6-phosphate dehydrogenase activity in sheep and goat erythrocytes (7). Recent experiments in this laboratory have shown that, in contrast to the results found in human cells, methemoglobin reduction in sheep erythrocytes is not accelerated by Nile blue sulfate, presumably because of a failure of dye linkage. This relative lack of stimulation by methylene blue in the pig and horse would suggest that the dye would be ineffective in the treatment of nitrite poisoning.

The results of these experiments correlate with clinical methemoglobinemia seen in veterinary practice. Cows, whose ability to reduce methemoglobin has not quite balanced the increased susceptibility of methemoglobin formation (Fig. 3), have the highest incidence of the disease. On the other hand, methemoglobinemia in swine is rarely reported and usually terminates rapidly in death, frequently without manifesting any external signs.

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SECTION III

ANNOTATED PAGES FROM THE DRAFT PROFILE DOCUMENT

ANNOTATED PAGES FROM DRAFT PROFILE
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Used in explosives	RDX is used as an explosive. It is a synthetic product that does not occur naturally in the environment (HSDB 2009).

1.2 WHAT HAPPENS TO RDX WHEN IT ENTERS THE ENVIRONMENT?

Found in water, soil, and air	RDX particles can enter air when it is disposed of by burning. RDX can enter water from disposal of waste water from ammunition plants. RDX and can enter water or soil from spills or leaks from improper disposal at plants or hazardous waste sites (Bohn et al. 1997; U.S. Army 1986a, 1986c, 1988).
Removal from soil, water, and air	RDX is slow dissolving in water. It does not bind significantly to soils and can leach to groundwater from soil. In water and air, RDX can break down in hours, but breaks down more slowly in soil. It does not build up in fish or people (Harvey et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1980c, 1984a).

CHANGE TO:

BIOACCUMULATE
BIOCONCENTRATE

1.3 HOW MIGHT I BE EXPOSED TO RDX?

Air	Typically, only people who work with RDX can potentially breathe RDX dust or get it on their skin. You can be exposed if you breathe fumes of burning RDX (Hathaway and Buck 1977; Kaplan et al. 1965; Testud et al. 1996a, 1996b).
Water and soil	You may be exposed to RDX by drinking contaminated water or by touching contaminated soil if you live near facilities that produce or use RDX. RDX has been found in water and soil near some ammunition plants and storage areas (Best et al. 1999; Kaplan et al. 1965; Simini et al. 1995; U.S. Navy 2005).
Food	RDX can be present in agricultural crops grown in contaminated soils irrigated with contaminated water (Harvey et al. 1997; Pennington and Brannon 2002; Simini and Checkai 1996).

1.4 HOW CAN RDX ENTER AND LEAVE MY BODY?

Enter the body **• Inhalation**	RDX can enter your body if you breathe in fumes of burning RDX or breath in the dust from powdered RDX (Kaplan et al. 1965; Testud et al. 1996a, 1996b).
• Oral	It can also enter the body if you drink water contaminated with RDX or accidentally or intentionally ingest explosives containing RDX (Davies et al. 2007; Harrell-Bruder and Hutchins 1995).
• Dermal contact	Much less RDX can enter the body through the skin (Reddy et al. 2008) if you come in contact with dusts of RDX or with liquids containing RDX.
Leave your body	Based on observations made in case reports (Woody et al. 1986) and results from studies in animals (Major et al. 2007; Schneider et al. 1977, 1978), most of the RDX is probably broken down rapidly in the body. These products, as well as unchanged RDX, are eliminated in the urine and exhaled air in a few days. RDX is not expected to accumulate in the body.

1.5 HOW CAN RDX AFFECT MY HEALTH?

Humans	If you breathe in dusts of RDX or intentionally or accidentally swallow large amounts of RDX, you may develop seizures (Davies et al. 2007; Kaplan et al. 1965; Küçükardalī et al. 2003; Testud et al. 1996a, 1996b). Some people exposed to large amounts of RDX also have alterations in blood pressure and in some components of the blood, but these effects may be secondary to the seizures. We do not know the effects of long-term, low-level exposure to RDX.
Laboratory animals	Animals that had large amounts of RDX placed in the stomach with a tube or that ate food mixed with RDX for longer periods of time suffered seizures (Burdette et al. 1988; Schneider et al. 1977; U.S. Army 1983a; U.S. Navy 1974b). Rats and mice that ate RDX for 3 months or longer had decreased body weights and slight liver and kidney damage (U.S. Army 1980b, 1983a). Rats that ate food containing RDX for 2 years had inflammation of the prostate (U.S. Army 1983a).

EXPLAIN BIOLOGICAL MEANING

INSERT: REPORTED

Cancer	There are no studies of cancer in people exposed to RDX. The EPA has determined that RDX is a possible human carcinogen based on the presence of liver tumors in mice that were exposed to RDX in the food for 1–2 years (U.S. Army 1984c).

1.6 HOW CAN RDX AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Effects in children	There are no studies of children exposed to RDX, but a child who accidentally ingested RDX had seizures (Woody et al. 1986), which is the same effect that occurs in adults exposed to high amounts of RDX. We do not know whether children are more susceptible to the effects of RDX than adults. We do not know whether RDX causes birth defects in humans.
Laboratory animals	Exposure of animals to RDX during pregnancy has not caused birth defects in newborn animals. However, rats exposed to RDX during gestation gave birth to slightly smaller babies than rats not exposed to RDX (U.S. Army 1986d). In rats exposed to RDX during pregnancy, RDX was able to pass through the placenta and reached the fetus (U.S. Army 2007b). Young deer mice (21 days old) were more sensitive than older deer mice (50 days old) to the acute toxic effects of RDX (Smith et al. 2007).
Breast milk	There are no studies that looked for RDX in human breast milk. However, rats exposed to RDX during pregnancy had RDX in their milk, suggesting that the same can occur in humans (U.S. Army 2007b). This means that women exposed to RDX who nurse their babies could pass RDX to the babies in the milk.

IS THIS LENGTH OR WEIGHT

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO RDX?

Consumer products	RDX is not found in consumer products. Therefore, families are not expected to have contact with RDX through the use of consumer products.

CHANGE TO: TRANSMIT

ANNOTATED PAGES FROM DRAFT PROFILE
SUBMITTED BY

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The absence of detectable hematological effects in rats in acute duration studies may relate to relatively high levels of methemoglobin reductase in their erythrocytes (Smith and Beutler 1966)

systemic end points were examined. Decreases in fetal weight and length were observed in the offspring of rats administered 20 mg/kg/day on gestation days 6–15 (U.S. Army 1986d); however, this exposure was associated with maternal convulsions/seizures and death.

Based on the available data, impaired neurological function was identified as the critical effect for derivation of an acute-duration oral MRL. Although the acute database lacks studies adequately assess systemic toxicity, intermediate-duration studies have not found systemic effects at doses lower than tho affecting the nervous system. The lowest adverse effect level for neurological effects is 12.5 mg/kg/da for decreases in motor activity and learning in rats following a single gavage dose (U.S. Army 1985b); this study did not identify a NOAEL. At a slightly higher dose (17 mg/kg/day), convulsions and tremo were observed in rats administered RDX for 14 days (U.S. Army 2006); no adverse effects were obser at 8.5 mg/kg/day. The U.S. Army (2006) study was selected as the principal study because it identified NOAEL and involved repeated exposure. In the U.S. Army (2006) study, groups of male and female Sprague-Dawley rats were administered via gavage 0, 2.125, 4.25, 8.5, 17.00, 25.50, 34.00, or 42.5 mg/kg/day as a suspension of RDX/1% methylcellulose/0.2% Tween 80 in distilled water 7 days/week for 14 days. Rats were monitored daily for toxic signs and morbidity. Body weights and feed consumption were measured on days 0, 1, 3, 7, and 14. Additional parameters used to assess toxicity included clinical chemistry and hematology values, organ weights, and gross necropsies. A significant increase in early deaths was observed at ≥25.5 mg/kg/day. Tremors and convulsions were observed in rats exposed to ≥17 mg/kg/day. In the males exposed to ≥17 mg/kg/day, blood stains around the mouth and nose and low arousal were also observed. High arousal, blood around the mouth and nose, barbering, and lacrimation were observed in females exposed to ≥17 mg/kg/day. No overt signs of toxicity were observed in rats exposed to ≤8.5 mg/kg/day. Significant decreases in body weight were observed in rats exposed to ≥8.5 mg/kg/day; however, the magnitude of these changes were not reported. Decreases in food consumption were also observed at these dose levels. Significant decreases in absolute liver weights and liver-to-brain weights and increases in blood cholesterol levels were observed in females exposed to 8.5 mg/kg/day; these effects were not observed at higher dose levels or in males.

Methemoglobin blood could be in case studies, MetHb Rats less sensitive - higher MetHb reductase

The acute-duration oral MRL was derived using the NOAEL/LOAEL approach; the lack of incidence data for the neurological effects precluded using a benchmark dose approach. The MRL of 0.09 mg/kg/day was calculated by dividing the NOAEL of 8.5 mg/kg/day for neurological effects by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

sensory reactivity to different types of stimuli), ophthalmic examination, urinalysis, clinical chemistry, hematology, coagulation, organ weights, gross necropsies, and histopathological examination of major tissues and organs from rats exposed to 0 or 15 mg/kg/day. Significant increases in mortality were observed at ≥10 mg/kg/day. Convulsions were observed in most animals dying early. Transient clinical signs included changes in arousal, inflammation of eyelash follicles, increased salivation, blood stains around mouth and nose, rough haircoat, tremors, and convulsions; the incidence and severity increased with dose. The incidences of convulsions were 0/20, 0/20, 3/20, 6/20, 13/20, and 12/20 in r[illegible] 0, 4, 8, 10, 12, and 15 mg/kg/day, respectively. Although the incidence of convulsions was statistically significant at 8 mg/kg/day, this dose level, which likely falls just below the NO[illegible] boundary, was considered a LOAEL due the seriousness of the effect. Neuromuscular effec[illegible] observed within the first week of exposure in the 12 or 15 mg/kg/day groups and persisted t[illegible] study. No significant RDX-related alterations in foot splay, front limb grip strength, or resp[illegible] stimuli were found. Hematological tests showed significant increases in erythrocyte mean cell volume at 8 (males only), 10, and 12 mg/kg/day and significant decreases in cholesterol levels in males exposed to ≥8 mg/kg/day. No significant increases in the incidence of histopathological alterations were observed.

Row 13 – Make clear that MRL is for org through the

The intermediate-duration oral MRL was derived using a benchmark dose (BMD) approach. As described in detail in Appendix A, the incidence data for convulsions in rats were fit to several dichotomous models using a benchmark response (BMR) of 10%; the log-probit model provided the best fit and was used to estimate a benchmark dose (BMD_{10}) of 7.01 mg/kg/day and a 95% lower confidence limit on the BMD ($BMDL_{10}$) of 5.31 mg/kg/day. The MRL of 0.05 mg/kg/day was calculated by dividing the $BMDL_{10}$ of 5.31 mg/kg/day for neurological effects by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Chronic-Duration

The chronic oral toxicity of RDX has been evaluated in two rat studies (U.S. Army 1983a; U.S. Navy 1976) and a mouse study (U.S. Army 1984c). A number of adverse health effects have been observed in rats exposed to 40 mg/kg/day including tremors, convulsions, and hyperresponsiveness; decreased hematocrit, hemoglobin, and erythrocyte levels; hepatomegaly and decreased serum cholesterol and triglycerides; renal papillary necrosis and increased blood urea nitrogen levels; testicular degeneration; and cataracts (females only) (U.S. Army 1983a). This dose was also associated with an 88% mortality rate. In addition to these effects, significant increases in the incidence of suppurative inflammation (see Table 2-1) were observed in the prostate of rats exposed to ≥1.5 mg/kg/day (U.S. Army 1983a). U.S.

three of five cases in which men accidentally ingested 37–250 mg/kg, an endoscopic examination conducted 3 days after exposure revealed erosive gastroduodenitis (Küçükardalī et al. 2003).

Vomiting was reported in dogs acutely exposed to 100 and 300 mg/kg/day RDX (Sunderman 1944) and in monkeys receiving gavage doses of 1 or 10 mg/kg/day for 90 days (U.S. Navy 1974b). Following intermediate exposure of rats to 50 mg/kg/day RDX, mild congestion of the intestines was reported (Sunderman 1944). No histopathology was seen in the stomachs or intestines of rats (Levine et al. 1981, 1990; U.S. Army 1980b, 1983a), mice (U.S. Army 1980b, 1984c), dogs (U.S. Navy 1974a; von Oettingen et al. 1949), or monkeys (U.S. Navy 1974b). Chronic exposure also did not produce histopathology in rats (U.S. Army 1983a; U.S. Navy 1976) or mice (U.S. Army 1984c).

Hematological Effects. Humans who accidentally consumed unknown levels of RDX for an acute duration generally had normal blood counts (Ketel and Hughes 1972; Woody et al. 1986). Temporary anemia and leukocytosis were reported in a study of six men who consumed unknown levels of RDX by using cooking utensils that were exposed to RDX fumes (Knepshield and Stone 1972). Similarly, leukocytosis and methemoglobinemia were noted in a report of five men accidentally ingesting 37–250 mg/kg RDX (Küçükardalī et al. 2003).

Rats less susceptible to MetHb reductase

Decreased hemoglobin and erythrocyte levels, increased platelet counts, and splenic extramedullary hematopoeisis were observed in male rats exposed to 40 mg/kg/day RDX in the diet for 6 months (U.S. Army 1983a). However, oral doses of 15 mg/kg/day (administered via gavage) (U.S. Army 2006) or 40 mg/kg/day (administered via the diet) (U.S. Army 1980b) for 13 weeks did not result in hematological effects. Similarly, decreased hemoglobin and erythrocyte levels were observed in mice exposed to 160 mg/kg/day for 90 days (U.S. Army 1980b). No hematological effects were found in mice exposed to 100 mg/kg/day for 6 months (U.S. Army 1984c) and dogs exposed to 50 mg/kg/day for 6 weeks (von Oettingen et al. 1949). Slight increases in the number of leukocytes were observed in rats exposed to ≥10 mg/kg/day for 13 weeks (Levine et al. 1981). Necrotic and degenerative megakaryocytes were observed in the bone marrow of monkeys given 10 mg/kg/day of RDX for 90 days (U.S. Navy 1974b). Chronic administration of 40 mg/kg/day of RDX in the diet for 1–2 years produced decreased hematocrit, hemoglobin, and erythrocytes in rats; the effects were considered slight and there were no compensatory responses (U.S. Army 1983a). Significant increases in platelet levels were also observed at 40 mg/kg/day (U.S. Army 1983a). No hematological effects were observed in mice chronically exposed to 100 mg/kg/day (U.S. Army 1984c).

Species differences in hematological responses to RDX may relate to differences in their activity of erythrocyte methemoglobin reductase (Smith and Beutler 1996; Rockwood et al. 2003).

RDX was extensively metabolized in rats (Schneider et al. 1977). Administration of a single gavage dose of 50 mg ^{14}C-RDX/kg resulted in <0.6% of the dose in the carcass 4 days after dosing and only 3% was excreted unchanged, mostly in the urine. The metabolites were not characterized.

A study of the metabolism of RDX in miniature pigs showed that RDX is rapidly and extensively metabolized by loss of two nitro groups followed by ring cleavage (Major et al. 2007). Pigs were administered a single gavage dose (43 mg/kg) of ^{14}C-RDX combined with carboxymethylcellullose in water and blood and excreta were collected for up to 24 hours. Metabolites were characterized by liquid chromatography/mass spectrometry (LC/MS) in selected samples of urine, plasma, and liver. Analysis of urine revealed two major metabolites, 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide. Using a more sensitive method of analysis, the investigators also identified MNX in both male and female urine and DNX in male urine. Analysis of plasma showed quantifiable amounts of RDX, and trace levels of MNX, DNX, and TNX. Analysis of liver extracts showed that most of the radioactivity was in the f of water-soluble, high-molecular-weight compounds rather than as RDX or any identifiable me

An *in vitro* study examining RDX metabolism (assessed by measuring loss of RDX) under low conditions determined that 46.6, 40.1, 34.6, 25.5, and 11.6% of the RDX was metabolized in hu monkey, pig, and rabbit microsomes, respectively, following a 30-minute incubation period (U.S 2008). After a 180-minute incubation period, 51.8, 47.2, 35.7, 33.7, and 18.0% of the RDX was metabolized, respectively. Under anaerobic conditions with nitrogen replacing oxygen, RDX was metabolized by several human recombinant cytochrome P450 isoforms (CYP1A1, CYP2B6, CYP2C8, CYP2C18, CYP2E1, CYP3A5); with the exception of CYP1A1, the RDX metabolite, MEDINA, was produced. In contrast, under aerobic conditions, no loss of RDX was detected in human liver microsomes, S9, hepatocytes, or a number of human recombinant cytochrome 450 isoforms (U.S. Army 2008).

RDX was metabolized *in vitro* by rabbit cytochrome CYP2B4 to 4-nitro-2,4-diazabutanal, nitrite, formaldehyde, and ammonia (Bhushan et al. 2003). This reaction was observed in a cell-free, isolated enzyme system; therefore, it's relevance to *in vivo* metabolism is unknown.

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

No relevant information was located from studies in humans or animals.

(U.S. Army 2007)

child. In this case, the model was not able to attain an acceptable fit using scaled parameter values. These findings suggest that the model is not adequately parameterized for metabolism or elimination o RDX, either because of use of a nonsaturable expression for hepatic metabolism or because of the absence of another significant (i.e., renal) elimination pathway.

I do not see human simulation in Krishnan et al 2009 paper. Need to cite Army 2007

Target Tissues. The model simulates concentrations of RDX in blood and brain, target tissues for RDX toxicity, as well as in blood, fat, and lumped compartments for slowly- and richly-perfused tissues.

Species Extrapolation. The model has been developed for simulations in rats and extrapolated to miniature swine and humans via allometric scaling of physiological flow rates and metabolism as a function of body weight. No data were presented to evaluate the model's ability to predict RDX levels in swine, while the model did not produce adequate reproduction of RDX in blood of a child ingesting 84 mg RDX/kg.

mice?

High-low Dose Extrapolation. The model has been evaluated for simulating inhalation exposures in mice and rats ranging from 500 to 5,000 ppm.

Interroute Extrapolation. The model simulates intravenous and oral exposures. Simulation of inhalation exposures would require additional parameterization; however, inhalation of RDX is not a significant route of exposure for risk assessment.

Strengths and Limitations. Strengths of the model are that it simulates disposition and clearance of intravenously injected or ingested RDX in rodents including predicting levels of RDX in the brain, a target tissue for toxicity. However, limitations include: (1) the uncertainty in the model to accurately simulate concentration-specific changes in metabolism and elimination rates in rats; (2) the model requires exposure-specific values to adequately simulate blood RDX in rats; (3) the model has not been evaluated against miniature swine data; and (4) the model has not been demonstrated to adequately predict RDX internal dose levels in humans.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. The mechanism(s) of absorption of RDX is not known. There are no studies that calculated rates of absorption that could have provided some indication of a possible mechanism of

absorption. In rats administered RDX in a capsule, peak blood concentrations were achieved 4–6 hours after dosing, which would indicate relatively low absorption rate (Crouse et al. 2008). In a male miniature pig given a single gavage dose of RDX as a suspension in 0.5% carboxymethylcellulose in water, peak plasma concentration of RDX occurred at approximately 12 hours after dosing, which would also suggest a relatively low rate of absorption. Studies with excised human and pig skin showed that mixing RDX with soil significantly reduced dermal absorption relative to RDX neat (Reddy et al. 2008; Reifenrath et al. 2002).

Distribution. No specific mechanism of distribution was apparent in the available studies. In rats, the distribution of RDX (single doses) seemed unaffected by the route of administration (parenteral vs. oral) or by the dose (Schneider et al. 1977). The concentration of RDX-derived radioactivity in most tissues was fairly stable between 2 and 24 hours after dosing except in the liver, where it fluctuated widely. High concentrations of radioactivity occurred in the liver at 2, 12, and 24 hours after dosing, which led Schneider et al. (1978) to suggest that there might be diurnal variations in the hepatic metabolism of RDX. In 90-day studies, RDX did not accumulate in any of the tissues examined (Schneider et al. 1978).

Metabolism. The metabolism of RDX has been studied in some detail miniature pigs (Major et al. 2007) and there is some evidence suggesting that a cytochrome, CYP2B4 orthologous to the rabbit may be involved. The two major metabolites characterized were 4-nitro-2,4-diazabutanal and 4-nitro-2,4-diazabutanamide. Trace amount of MNX, DNX, and TNX were also detected. Some studies have provided some information regarding the role of metabolism in the toxicity of RDX. In rats, administration of RDX intravenously resulted in convulsive activity within seconds after the injection, which suggested that the convulsions are caused by the parent compound (Schneider et al. 1977). In a 90-day gavage study in monkeys, convulsive events were associated with higher RDX concentrations in plasma (U.S. Navy 1974b), which would also support the idea of the parent compound being responsible for the convulsive activity. More recently, Meyer et al. (2005) reported that MNX and RDX were equipotent in causing convulsions and lethality in female Sprague-Dawley rats in single dose of 14-day duration gavage studies; both DNX and TNX were less potent. In a study of age-dependent acute toxicity of RDX in deer mice, Smith et al. (2007) reported that, for all three age brackets tested, RDX was significantly more potent than MNX and TNX.

Excretion. The urine and exhaled CO_2 were the main routes of excretion of ^{14}C-RDX-derived radioactivity in rats following acute- or intermediate-duration exposure to RDX (Schneider et al. 1977, 1978). In the acute studies, only 3% of the administered radioactivity was recovered in the feces over a 4-day period (Schneider et al. 1977). The urine was also the main excretory route of radioactivity in

Nomenclature CYP2B4 is the rabbit isozyme of pig? Basis for rabbit

Space in 14d Not 14d dose gavage Single dose observe

miniature pigs following a single gavage dose of RDX (Major et al. 2007). No information was located regarding how the size of the dose might affect the distribution of metabolic products among excretory pathways.

3.5.2 Mechanisms of Toxicity

The main effect of high doses of RDX in humans and animals is the induction of hyperactivity manifested as convulsions or seizures. RDX has also induced other effects; however, because these effects have not been well characterized and/or have been seen inconsistently in animal studies, this section will focus mainly on the potential mechanisms of neurotological effects. Hyperactivity can result from a chemical acting centrally and/or on the peripheral nervous system. Chemicals such as organophosphorous pesticides or nerve ~~gets~~ gasses such as sarin and soman act mainly by inhibiting cholinesterase activity in the brain (McDonough and Shih 1997), but no information is available regarding possible effects of RDX on cholinesterase activity. Based purely on the chemical structure of RDX, it seems unlikely that it would have strong anticholinesterase properties. In rats receiving a single intraperitoneal dose of RDX, small, but significant, decreases in brain cholinesterase levels were found 1.5, 3, or 6 hours after dosing. By 24 hours after dosing, the cholinesterase levels were similar to controls (Maryland University 1975). However, in rats receiving 2.5 or 6.5 mg/kg/day RDX administered intraperitoneally for 6 or 12 weeks, significant increases in brain cholinesterase levels were found. An *in vitro* study found a 53% decrease in cholinesterase activity in brain homogenates incubated with 4.5×10^{-3} M RDX (Maryland University 1975). The Maryland University study (1975) also found significant increases in monoamine oxidase activity in rats receiving intraperitoneal doses of 2.5 or 6.5 mg/kg/day RDX for 6 or 12 weeks or 0.3 mg/kg/day for 12 weeks. However, following a single dose, a small nonsignificant decrease in monoamine oxidase activity was observed 0.5, 1.5, 3, 6, or 24 hours after dosing. As with cholinesterase activity, RDX induced a dose-related decrease in monoamine oxidase activity *in vitro*.

Another possibility is the involvement of GABA (γ-aminobutyric acid) receptors in RDX-induced neurologic dysfunction. Antagonism of GABAergic neurons within the central nervous system leads to generalized nervous system stimulation. Binding of GABA to its receptor opens chloride-selective ion channels leading to influx of chloride into neurons through an electrochemical gradient resulting in hyperpolarization of the membrane and inhibition of cell firing. A reduced inhibitory drive results in uninhibited activity in effector neurons. However, since no studies have been conducted to test this hypothesis, the involvement of GABA or any other transmitter system remains pure speculation at this time. Some support for the hypothesis of an RDX-induced imbalance between inhibitory and excitatory

axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et a 1993; Hoel et al. 1992).

Why only the in vitro studies here? Earlier ref to testicular effect

No *in vitro* studies were located regarding endocrine disruption of RDX.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The nervous system is one of the main targets for RDX toxicity in humans exposed by the inhalation (Hollander and Colbach 1969; Testud et al. 1996a) or oral (Goldberg et al. 1992; Harrell-Bruder and Hutchins 1995; Hollander and Colbach 1969; Ketel and Hughes 1972; Knepshield and Stone 1972; Küçükardalï et al. 2003; Merrill 1968; Stone et al. 1969; Woody et al. 1986) routes, and animal studies involving oral exposure support this finding (Burdette et al. 1988; Meyer et al. 2005; Schneider et al. 1977; U.S. Army 1985b, 2006). There is a small number of acute-duration animal studies and no studies adequately examined for potential systemic effects. Increases in occurrence of convulsions, tremors, and/or seizures were consistently observed in the available studies (Burdette et al. 1988; U.S. Army 1980b, 1985b, 1986d, 2006). In addition, decreases in growth were observed in the fetuses of rats exposed to lethal doses of RDX (U.S. Army 1986d). One animal study suggests that the skin is a target organ for RDX following dermal exposure (U.S. Army 1974). However, the use of solvents confounded the results. No acute inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. The available acute exposure data for animals was adequate for the derivation of an MRL based on an increased incidence of convulsions/seizures/tremors (U.S. Army 2006). Further acute inhalation and oral studies on the developmental and neurological effects of RDX would be useful in determining levels that may cause harm to humans living near hazardous waste sites; these studies should also evaluate potential systemic effects.

Intermediate-Duration Exposure. No studies examining the toxicity in humans following intermediate-duration exposure to RDX were identified. No animal studies were identified examining RDX toxicity following inhalation exposure; thus, an intermediate-duration inhalation MRL could be derived. Inhalation studies are needed to identify potential targets of toxicity and establish dose-response relationships; these studies would be useful in determining levels that may cause harm to humans who live near hazardous waste sites. The nervous system is the target organ for RDX toxicity in animals exposed by the oral route for intermediate periods (Levine et al. 1981, 1990; U.S. Army 1983a, 1985b, 2006; U.S. Navy 1974b; von Oettingen et al. 1949). The most consistently observed effect was convulsions, seizures, and tremors. Systemic effects (hematological and serum chemistry alterations), reproductive effects (testicular degeneration, possible decrease in male fertility), and developmental effects (decreases in growth and decreased viability) have also been observed. However, these effects have not been consistently observed across studies. Difference in the exposure route (dietary versus gavage) and RDX formulation (finely ground versus coarsely ground) may explain possible differences in

not Could MRL be derived?

the results; however, this has not been adequately assessed and additional oral exposure studies are needed to evaluate apparent study differences. An intermediate oral MRL based on an increased incidence of convulsions in rats was derived (U.S. Army 2006). Studies involving intermediate dermal exposure to RDX did not identify a target organ (U.S. Army 1974).

Chronic-Duration Exposure and Cancer. Only one human study was located for chronic-inhalation exposure. This study revealed no adverse health effects following chronic exposures to unknown levels of RDX in the air (Hathaway and Buck 1977). No animal studies concerning chronic inhalation exposure were located. No chronic inhalation MRLs could be derived because of the lack of human and animal studies with accurate exposure estimates. Therefore, further inhalation studies would be useful to identify target organs and define the potential for human health risks.

No human studies concerning chronic oral exposure were located. The most sensitive target organ for adverse effects in animals following chronic oral exposure has not been well defined. Chronic-duration oral animal studies provide information regarding mild adverse systemic effects in rats (U.S. Army 1983a) and mice (U.S. Army 1984c). The other significant adverse effect found was an increased occurrence of convulsions and seizures in rats (U.S. Army 1983a). A second chronic-duration study in rats (U.S. Navy 1976) did not find any adverse effects. An increased incidence of prostate gland inflammation was observed in rats exposed to RDX for 2 years (U.S. Army 1983a). The inflammation was observed at the lowest adverse effect level; it may have been secondary to a bacterial infection. Because the second rat study (U.S. Navy 1976) did not examine the prostate, the prostate effect could not be confirmed. Additional studies are needed to further evaluate the prostate as a potential target of RDX toxicity. A chronic-duration oral MRL was not derived for RDX; due to the uncertainty whether the prostate effect was due to RDX exposure, this end point was not considered a suitable basis for an MRL. Basing the MRL on the next highest adverse effect level resulted in an MRL that was higher than the intermediate-duration oral MRL. Only one human study was located for chronic dermal exposure (Sunderman 1944). This study reported dermatitis in workers exposed to RDX, but no dose levels were reported. No animal studies concerning chronic dermal exposure were located. Additional chronic oral and dermal studies would be useful to better define dose levels that may cause a risk to humans.

Prostate Study should include assessment of immuno-toxicity.

these studies should include endpoints addressing immunotoxicity of chronic-duration RDX exposure.

No studies are available regarding cancer in humans following any route of exposure. Increased incidences of combined hepatocellular adenomas and carcinomas were found in female mice orally exposed to RDX (U.S. Army 1984c). A re-evaluation of the histopathology slides from this study resulted in a re-classification of several of the tumors as nonneoplastic alterations (Parker et al. 2006). No

increases in neoplastic lesions were observed in rat oral exposure studies (U.S. Army 1983a; U.S. Navy 1976). The risk of developing cancer by the inhalation or dermal routes has not been investigated. Further inhalation, oral, or dermal carcinogenicity studies would be useful to determine whether RDX poses a risk of cancer for humans.

Genotoxicity. Data from microbial mutagenicity studies using *S. typhimurium* and *S. cerevisiae* have consistently produced negative results (George et al. 2001; Lachance et al. 1999; Pan et al. 2007; U.S. Army 1977b, 1980b; Whong et al. 1980). Therefore, at this time, additional studies with RDX would probably not provide any new key information. Studies involving humans and mammalian species are few. The three mammalian studies available were negative for DNA damage in human fibroblasts (U.S Army 1978b), dominant lethal mutations in rats (U.S. Army 1980b), and induction of micronuclei in bo marrow cells from mice (Reddy et al. 2005a). Epidemiological studies involving humans exposed occupationally or militarily to RDX may help to confirm its status as a human genotoxin.

Additional data – RDX metab. MNX, TNX

Reproductive Toxicity. No data are available on the reproductive toxicity of RDX in humans via inhalation, oral, or dermal routes of exposure. No inhalation or dermal studies are available for animals. An oral study in mice (U.S. Army 1984c) and one in rats (U.S. Navy 1976) revealed no histopathology in the ovaries, testes, or uterus. One oral study (U.S. Army 1983a) did reveal spermatic granulomas in the prostate of rats after 6 months of exposure and testicular degeneration in rats exposed for 1 year. This study also reported an increased incidence of suppurative inflammation of the prostate in rats exposed for 2 years; however, the inflammation was primarily observed in rats dying early and there is concern that the inflammation may be secondary to a bacterial infection rather than a primary effect of RDX. No pharmacokinetic data are available that can be used to determine whether the reproductive system is likely to be a target for RDX toxicity. Therefore, further studies to determine whether the prostate is indeed the most sensitive organ are important. A two-generation reproductive study in rats (U.S. Army 1980b) reported nonsignificant decreases in F_0 male fertility when the exposed males were mated with unexposed females or exposed females; additional studies are needed to confirm this effect.

Developmental Toxicity. No human studies on developmental effects are available for exposure to RDX via inhalation, oral, or dermal routes. No inhalation or dermal studies are available for animals. Two acute duration oral studies examined the potential developmental toxicity of RDX. Maternal deaths were observed in both studies at the highest dose tested (U.S. Army 1980b, 1986d). No increases in the occurrence of fetal malformations were observed (U.S. Army 1980b). One study reported a decrease in fetal weight and length at the dose level associated with maternal deaths and neurotoxicity. In a two-

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

RDX has been identified at 31 out of the 1,699 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2007). However, the number of sites evaluated for RDX is not known. The frequency of these sites can be seen in Figure 6-1.

RDX is a military explosive produced by the nitrolysis of hexamine with nitric acid (Boileau et al. 2009). It is a synthetic compound and is not known to exist in nature. Effluents and emissions from ammunition plants are responsible for the release of RDX into the environment (Pennington and Brannon 2002; U.S. Army 1984a). RDX is expected to exist as a particulate in the atmosphere. When released to water, RDX is subject to photolysis (half-life of 9–13 hours). Photoproducts include formaldehyde and nitrosamines (U.S. Army 1980a). Alkaline hydrolysis can also occur (Balakrishnan et al. 2003; Heilmann et al. 1996). RDX undergoes biodegradation in water and soil under anaerobic conditions (Funk et al. 1993; Pennington and Brannon 2002; U.S. Army 1984f). Its biodegradation products include ~~hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine~~ MNX; DNX; TNX; hydrazine; 1,1-dimethyl-hydrazine, 1,2-dimethyl-hydrazine; formaldehyde; and methanol (McCormick et al. 1981). RDX is mobile in soil, and can leach into groundwater (U.S. Army 1980c), and can be transported from soils or water to terrestrial and aquatic plants (Best et al. 1999; Harvey et al. 1991, 1997; Pennington and Brannon 2002; Simini and Checkai 1996).

Abbrev for MNX has been defined p34

RDX has been identified in environmental samples, primarily near army munition depots (Bishop et al. 1988; Dacre 1994). Indoor air samples collected at ammunition plants were found to contain RDX in concentrations ranging from 0.032 to 60 mg/m^3 (Bishop et al. 1988; U.S. Army 1975). In water, RDX has been identified in a variety of groundwater samples from ammunition plants in the United States (<1–14,100 µg/L) and Germany (21–3,800 µg/L) (Bart et al. 1997; Best et al. 1999; Godejohann et al. 1998; Steuckart et al. 1994; U.S. Army 1988). Sediment samples from Army depots have been found to contain RDX in concentrations ranging from <0.1 to 3,574 mg/kg (Simini et al. 1995; Sunahara et al. 1999; U.S. Army 1988) and in composts prepared from contaminated sediments (>2.9–896 mg/kg) (Griest et al. 1995; Gunderson et al. 1997). Additionally, RDX was indentified in plant species irrigated with or grown in contaminated water (<20–3,196 µg/L) (Best et al. 1999; Pennington and Brannon 2002).

use consistent nomenclature 1443 ppb

Ammunition Plant near Milan, Tennessee contained RDX at a concentration of 1,443 µg/L. Filtration reduced RDX concentration in the water samples by 27% (Best et al. 1999). Groundwater samples fron the Umatilla Army Depot Activity, a munitions storage and handling depot in Hermiston, Oregon and th Naval SUBASE Bangor in Bangor, Washington contained RDX in concentrations ranging from <20 to 8,160 ppb (Bart et al. 1997).

Groundwater samples from monitoring and extraction wells at the Naval Base Kitsap at Bangor NPL site at the Naval Base Kitsap at Bangor in Kitsap County, Washington, were collected from May 1994 to August 2004. Concentrations of RDX in the samples from a 12-acre Bangor Ordnance Disposal site (Site A) ranged from 0.19 to 1,000 µg/L in perched zone monitoring wells, from 0.19 to 550 µg/L in shallow aquifer monitoring wells, and from 0.4 to 660 µg/L in extraction wells (shallow aquifer). RDX concentrations at the site of a former waste water lagoon and overflow ditch (Site F) in groundwater from a shallow aquifer ranged from 0.95 to 3,800 µg/L (U.S. Navy 2005).

RDX was identified in environmental samples at Cornhusker Army Ammunition Plant and Louisiana Army Ammunition Plan army bases (Dacre 1994). Maximum concentrations of RDX detected in water at the Cornhusker Army ammunition plant (Nebraska) were 0.307 and 0.371 ppm from on- and off-site wells, respectively (Agency for Toxic Substances and Disease Registry 1989a). A plume of RDX-contaminated groundwater, which stretched 6.5 km, was found near the Cornhusker Army ammunition plant. The concentrations ranged from 9 to >100 µg/L (Spalding and Fulton 1988). The Louisiana Army ammunition plant is a shell manufacturing and explosives load, assembly, and pack facility (U.S. Army 1988). From 1951 to 1980, waste waters were trucked to and discharged into a series of artificial leaching pits, which resulted in contamination of soil, sediments, and groundwater. Levels of RDX measured in groundwater at the Louisiana Army ammunition plant ranged from 1.3 to 14,100 µg/L (U.S. Army 1988).

RDX was identified in a water sample obtained from a military training site in Germany at 21 µg/L (Godejohann et al. 1998). Two contaminated water samples from the area of a former explosive production plant at Elsnig in Saxony, Germany contained RDX at concentrations of 2,380–3,800 and 310–400 µg/L, with the exact concentrations dependent upon the method of detection (Steuckart et al. 1994).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children residing in areas around Army ammunition plants where RDX is manufactured, converted to munitions, or released through the demilitarization of antiquated munitions may be exposed to RDX (Hundal et al. 1997; Pennington and Brannon 2002; U.S. Army 1980a, 1984a, 1984f). The primary route of exposure is ingestion of contaminated drinking water. Inhalation exposure may result from breathing contaminated particulate matter produced during incineration of RDX-containing waste material. Dermal contact with contaminated soil is also a possible route of exposure. Children playing in contaminated water or soil may also be exposed via ingestion.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Workers involved in the production and use of RDX at Army ammunition plants constitute a group at risk because of the potential for occupational exposure. Persons living near Army ammunition plants or hazardous waste sites may have a higher risk of exposure to RDX resulting from inhalation of dusts or fumes, ingestion of contaminated drinking water, or contact with contaminated soil (Hundal et al. 1997; Pennington and Brannon 2002; Testud et al. 1996b). Military personnel may also be exposed to high levels from the use of explosives that contain RDX.

What about dumping/disposal of nuclear & bio chemical weapons under treaty?

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1980d - out of order

out of order

Table 8-1. Regulations and Guidelines Applicable to RDX

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	No	IARC 2009
WHO	Air quality guidelines	No	WHO 2000
	Drinking water quality guidelines	No	WHO 2006
NATIONAL			
Regulations and Guidelines:			
a. Air			
ACGIH	TLV (8-hour TWA)[a]	0.5 mg/m^3	ACGIH 2008
	STEL (15-minute TWA)	No	
	TLV-basis (critical effect)	Liver damage	
AIHA	ERPG values	No	AIHA 2008
EPA	AEGL values	No	EPA 2008a
	Hazardous air pollutant	No	EPA 2009b 42 USC 7412
NIOSH	REL (10-hour TWA)	1.5 mg/m^3	NIOSH 2005
	STEL (15-minute)	3.0 mg/m^3	
	IDLH	Not determined	
	Target organs	Eyes, skin, and central nervous system	
OSHA	PEL (8-hour TWA) for general industry	Vacated[b]	OSHA 1993 29 CFR 1910.10 Final Rule
b. Water			
EPA	Drinking water standards and health advisories		EPA 2006a
	1-day health advisory for a 10-kg child	0.1 mg/L	
	10-day health advisory for a 10-kg child	0.1 mg/L	
	DWEL	0.1 mg/L	
	Lifetime	0.002 mg/L	
	10^{-4} Cancer risk	0.03 mg/L	
	National primary drinking water standards[i]	No	EPA 2003
	National recommended water quality criteria	No	EPA 2006b
c. Food			
FDA	EAFUS	No[c]	FDA 2008

What is superscript i"

For national 10 drinking water standard?

RDX is on 2003 CCL 2 list

What is "i" superscript?