Department of Defense (DOD) Comments on the Interagency Science Consultation Draft IRIS Assessment of RDX (dated September 2014)

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Department of Defense Comments on RDX_Interagency Consultation draft Toxicological Review_9-30-14.pdf		
Comments submitted by: Chemical Material Risk Management Directorate	Organization: Department of Defense	Date Submitted: 10/27/2014
*Comment categories: Science or methods (S); Editorial, grammar/spelling, clarifications needed (E); or Other (O). Also please indicate if Major i.e. affects the outcome, conclusions or implementation of the assessment.		

Comment No.	Section	Pages	Comment	Suggested Action, Revision and References (if necessary)	*Category
1	Executive Summary	ES-1	Line 5: “Increased mortality was generally observed at RDX doses that induced nervous system effects” is very misleading to the reader. In over 60 years of RDX in manufacturing and use, even during times when occupational or environmental regulation was not well established, there has not been a single reported fatality case due to inadvertent exposure in humans or animals. In 12 published cases of human overexposure to RDX, there has not been one documented case of mortality. Additionally, in animal studies it is not completely clear if seizure=mortality in all studies.	DoD suggests that at a minimum, this sentence lead off with “In some animal studies, increased mortality was generally…” Also, not comments regarding the evaluation of mortality/lethality as an independent endpoint, and the need for quantitative evaluation of mortality associated with seizure.	S
2	Executive Summary	ES-1	Here the EPA suggests that the mechanistic data are insufficient to establish a Mode of Action when the following sentence presents the mechanism. This is contradictory.	We suggest that EPA state that the mechanism is the disruption of the chloride channel initiated through RDX affinity to the picratoxic site of the GABA-alpha site and that	S

				the mode of action is quick oral absorption and distribution of RDX through the blood-brain barrier that causes convulsions via disruption of neuronal chloride homeostasis.	
3	Executive Summary (and elsewhere)	ES-2, line 20-23, ES-5, line 11-12 and elsewhere	DoD does not agree that "severity" of an endpoint is a criterion for changing either the BMR or the uncertainty factors, as delineated in existing EPA guidelines and guidance. DoD feels that using a BMDL 1% using animal (not epidemiological) data is counter to USEPA's BMDR guidance cited in this document. ES-5, lines 5-8 states "U.S. EPA (2012a) emphasizes that when modeling a dose-response relationship from a given set of data, statistical and biological characteristics of the dataset must be considered, including consideration of the severity of the effect. A key consideration in this assumption is that seizure is equivalent to mortality. Crouse et al. (2006) has provided animal specific information that shows this assumption is incorrect (see additional information made available by the study authors, provided by DoD to EPA in the attached "Crouse_2006_MortalityFrequencyTable1.doc"). Additionally, this assumption is NOT supported by the human accounts that report seizure and neurological effects but not mortality. Simply put, calculating a BMDL (i.e. 95% lower confidence interval) at the 1% implies that rats are the same as humans, bolus is the same as incremental oral exposures, and that seizure is equivalent to death, all of which are not supported by the data. On pg ES-2, line 20-23 it states "A 1% response level was chosen because of the severity of	Reconsider using a BMDL of 10%, consistent with established EPA guidance and practice. See additional comments on Section 2.1.4.	S/M

			the endpoint; this is supported by the observation in Crouse et al (2006) that for all dose groups…mortality was strongly associated with convulsions." Given that mortality in humans has never been recorded, even at very high doses, the 1% response would seem to be over-reaching. Furthermore, extrapolation below the data assumes (contrary to the noncancer standard assumption) that there is no threshold below which convulsions would not be observed, i.e., that the 10% response rate is predictive quantitatively of the 1% response rate. The document, however, notes (see, for example, page 1-2) that there were several experiments where the doses were too low to produce convulsions, e.g., "No evidence of seizures, convulsions or tremors was reported in three subchronic rat studies that used relatively lower doses of RDX (highest administered doses: 10-50 mg/kg-day)". We agree with EPA on ES-5 line 11-12, "Use of a BMR of 1% extra risk of convulsions resulted in extrapolation below the range of experimental data and could potentially increase uncertainty in the BMD and BMDL values." In sum, DoD feels that the use of the BMR 1% adds unnecessary uncertainty, is inappropriate if mortality is a concern as this endpoint can be evaluated independently, is technically unfounded given than seizures/convulsions have not lead to mortality in any human cases, and is contrary to EPA guidance.		
4	Executive Summary, Evidence for	ES-2	There is conflicting evidence supporting the contention that RDX is a human carcinogen that precludes quantifying a slope factor to assess the carcinogenic	Consider the entire weight of evidence and conclude that the data do not support a	S/M

	Human Carcinogenicity		risk from RDX exposure. The mutagenicity and genotoxicity data for RDX are negative. The chronic rat study found no statistical differences in lung tumors between treatments. The chronic mouse study (Lish et al. 1984; reevaluated by Parker et al. 2006) found no statistical differences between treatments in combined adenomas and carcinomas in male mice. Only for females where statistical differences were found between controls and mid-dose treatments; however, no differences were found between controls and high dose female treatments suggesting a lack of a dose response relationship. There is no plausible reason for the observed differences between the sexes. Additionally, the oral exposure dose was changed partway through the study from an estimated 175 mg/kg (where mortality was reported) to 100 mg/kg-d suggests the Maximum Tolerated Dose was exceeded and by changing dose imparts a high degree of uncertainty regarding exposure (Table 2-6 needs to show this). Although a time weighted average was calculated, the EPA cannot show if the cancer incidence was due to a latent effect from the initial high dose. Moreover, the background incidences of cancer in the control animals were below historical levels for this strain of mouse. The trend statistical analyses were conducted without presentation of the power or variation (least squares error, or R2-value) to enable any objective interpretation. This combined weight of evidence suggests any quantifying of cancer risk to humans is ambiguous at best, that the association could easily be explained by chance (Type	quantitative estimate of cancer risk for humans at this time.	

			I Error), and provides little to support to the derivation of cancer risk calculation, particularly when, in effect, will be used by other state regulatory agencies to enforce remedial standards under the likely false perception that they are protecting public health.		
5	1.1.1	1-1 to 1-3	Describing the variation in the observations of seizure incidence is best described by differences in the absorption, distribution, metabolism and excretion of RDX. The chronic studies where RDX was provided in feed calculated daily exposures as high as 100 mg/kg-d with few reported seizure events; however, gavage studies frequently reported seizure at oral exposures at relatively an order of magnitude lower. Crouse et al. (2006) describe that seizure was most often observed directly following dosing which suggests that there is an important issue associated with rats receiving an oral bolus via gavage which kinetically is quite different than an incremental daily (24-hr) exposure scenario suggested through this application in the calculation of a RfD. This obvious point suggests RDX is absorbed rapidly following oral exposure and that seizure was best described by peak brain/plasma RDX concentrations (see Bannon et al. 2009, Burdette et al. 1988, Williams and Bannon 2009, and Williams et al. 2011); suggesting that peak rather than AUC RDX concentrations is the most appropriate dose metric for the PBPK model (see Sweeney et al. 2012a).	DoD recommends that EPA consider the weight of evidence describing the etiology of RDX-induced convulsive episodes described in the animal, human, and focused animal and in vitro data that sufficiently describe the MOA and mechanism. Importantly, consider peak plasma/brain concentrations that initiate seizure and the differences in the biokinetics between feeding studies and gavage (bolus) exposure regimes. Combined, the evidence indicates that peak rather than AUC RDX concentrations is the most appropriate dose metric for the PBPK model.	S/M
6	1.1.1	1-2	With respect to the paper by Zhang and Pan (2009a) the letter commentary by Bannon (2009) which	Please include correspondence between Bannon and Zhang regarding this publication	S

			showed that "The dose used by Zhang and Pan in their 1-month study was therefore less than the lowest dose in the 2-year mouse cancer study and over 20 times lower than the only dose of RDX associated with cancer". It is inaccurate to show the concentration in the food (5 mg/kg-day) as a dose when actual conversion to body weight would lead to a maximum dose of 1.5 mg/kg-day. The subsequent discussion in correspondence has not been captured in the literature review.	by citing. Bannon DI, Johnson M, Williams L, Adams V, Perkins E, Gust K, Gong P. RDX and miRNA Expression in B6C3F1 Mice. Environ Health Perspect. 2009 Mar;117(3):A98;author reply A98-9. Accordingly, correct the dose to the body weight adjusted 1.5 mg/kg-day.	
7	1.1.1	1-5	Table 1-1. There are many other accounts of human exposures to RDX that are not captured in this table. Although they may fail to have accurate exposure estimation, the description of symptoms and sequelae are important evidence in understanding the relevance of laboratory animal extrapolation of endpoints and data.	Include human accounts summarized in a table. These accounts will help provide the weight of evidence necessary to infer from the animal data on the uncertainty associated with animal to human extrapolation.	S
8	1.1.1	1-5	Table 1.1: It would appear from the evidence presented in Table 1.1 that there were many animal studies where seizures were not reported. In the chronic feeding studies of Lish (1984), Hart (1976), and Levine (1983) some seizures were reported at the highest doses (35-100 mg/kg) but not at intermediate or lower doses. The Crouse study was a daily 90-day gavage study, and seizures at lower doses may well have been due to the bolus effect (sudden peak of RDX after dosing), since the peak RDX levels in blood and brain are the best internal predictor of seizure.	Consider a way to graphically present the negative evidence for seizure in RDX feeding studies. Consider discussing these data as a means for identifying a threshold for nervous system effects.	S

9	1.1.1	1-12	After Figure 1-1 on page 1-12, pagination starts over with a second Page 1-1.	Please correct pagination of Section 1.	E
10	1.1.1	1-12	Figure 1-1. Footnote 3 "Due to the severity of the endpoint for convulsions and/or seizures, a response in treated groups was determined to be significant (filled circles) in the exposure-response array where there was an observation of convulsions and/or seizures reported in the study." If the data were not statistically significant from the controls, the response cannot be judged positive because the evaluator deems the response "significant".	The filled dots should not be labelled "significantly changed" in the absence of statistical significance. If a response is not “statistically significant”, the response incidence is NOT different from controls. If the response is “statistically significant”, then a case for (or against) biological significance can then be made after comparing incidence with normal ranges for this laboratory animal species and relevance of endpoint given the differences in physiology between the model and humans. Suggest striking this language as the response is not statistically significant.	S/M
11	1.1.1	1-2 (second instance of this page number) 1-1	Line 35: EPA concluded that “the available data are insufficient to identify any specific mode(s) of action for the nervous system effects observed following RDX exposure.” action is highly plausible and well-supported by mechanistic data. EPA provided a great deal of discussion into explaining the kidney and urogenital effects using the GABAα receptor, however, DoD feels there was insufficient discussion of the validity of the GABAα mechanism as the underlying cause of seizures. For example, the fact that benzodiazepines, therapeutically effective in RDX-induced seizures, act at the GABAα receptor is an important observation that supports this mode of action. This was not adequately discussed in identification of hazard.	We suggest that EPA reconsider the mode of action for seizures mediated via RDX binding to GABAα. Reconsider the importance and the impact of the proposed GABAα mode of action in this document. Discuss the weight of evidence describing the etiology of RDX-induced convulsive episodes described in the animal, human and focused animal and in vitro data that sufficiently describe the MOA and mechanism.	S

12	1.1.1 Mechanistic Evidence.	1-17	While a great deal of discussion has gone into attempting to explain the kidney and urogenital effects using the GABAα receptor, there was insufficient discussion of the validity of the GABAα mechanism as the underlying cause of seizures. For example the fact that benzodiazepines, therapeutically effective in RDX-induced seizures, act at the GABAα receptor is an important observation that supports the mechanism of action. This was not discussed in identification of hazard.	More discussion of the relevance of the proposed GABAα receptor mechanism is needed as a probable and supported Mode of Action for neurological effects.	S
13	1.1.1. Nervous System Effects	1-2 to 1-3	"In general, gavage dosing (Crouse et al.,2006; Cholakis et al., 1980) induced convulsions at lower doses than did dietary administration, possibly due to the bolus dosing resulting from gavage administration and the comparatively faster peak absorption of RDX." DoD agrees that since the Crouse study was a daily 90-day gavage study, seizures at lower doses may well have been due to the bolus effect (sudden peak of RDX after dosing), since the peak RDX levels in blood and brain are the best internal predictor of seizure. Table 1.1 shows several animal studies where seizures were not reported. In the chronic feeding studies of Lish (1984), Hart (1976), and Levine (1983) some seizures were reported at the highest doses (35-100 mg/kg) but not at intermediate or lower doses. Despite the noted differences in results based on RDX administration, and the fact that people will not be exposed to RDX by gavage, the gavage data was utilized for quantitative analysis without accounting for this greater sensitivity/effect. Furthermore, EPA added additional quantitative	To provide a more thorough and transparent assessment of the seizure endpoint, consider adding additional discussion that highlights the negative evidence for seizure in RDX feeding studies, which should include a more thorough discussion exploring the differences between the dose metric for gavage and dietary administration of RDX, and considering the scenario most relevant to human health. The human data are a weight of evidence that suggests seizure incidence is not equivalent to death. Given that convulsions were seen at lower doses by a route of exposure unlikely for people, and dietary studies demonstrate the more relevant dose-response for human exposure, DoD feels that EPA should either use a different dose-metric (i.e. peak plasma concentration) or not use the data from the Crouse study in this manner. At a minimum, EPA should provide justification for utilizing Crouse et al.	S/M

			manipulations (e.g. using a BMR of 1%) to the gavage study that that further complicated this extrapolation in a direction that was not scientifically supported (extrapolation to humans from repetitious, chronic, daily exposures). DoD feels that the available information suggests that peak plasma concentration is important in seizure development, incidence of seizure is not equivalent to mortality (evidenced by lack of death in humans where seizure incidence was reported and in the rodent data) and that using a 1% BMDL is not supported given the variation and complicating issues extrapolating the data.	as the key study, and should not use the 1% BMDL as this suggests a level of precision that is not represented in the data. DoD believes that the bolus dosing regimen combined with the low BMR is unnecessary compounding conservatism.	
14	1.1.3	1-21	If changes (reductions in fertility; decreased number of pregnancies) were not statistically significant, they are NOT DIFFERENT. It is incorrect to state that there were differences if not statistically shown to be so. This is done throughout the document (see Pp. 1-51, line 1).	Do not refer only to mean values to represent differences if they are not statistically different.	S
15	1.1.3.	1-24	DoD commends EPA for clearly distinguishing which statistical analyses were performed by the authors of the study and which were performed by EPA personnel.	DoD strongly encourages EPA to continue this practice in other assessments.	S
16	1.1.4	1-32	Lines 12-16: EPA considered changes in clinical chemistry parameters statistically significant as compared to the control mean. However, this may not be biologically meaningful if not outside the normal ranges for these parameters, for these species. The evaluation of biological significance has not been	Statistical significance and biological significance are two different things. Use statistical significance to discuss differences in treatment mean values (or medians). Use ranges of normal values to determine if adverse health events are biologically	S

			done. This analysis was done, however, for the human data from Hathaway and Buck (1977) in table 1-10).	plausible (not unlike human health assessments).	
17	1.1.5	1-45	Here, the text reports a lack of evidence that bronchiolar/alveolar adenomas or carcinomas are related to treatment. This appears to contradict the text stating that they are dose-related (Pp. 1-47, lines 22-24).	Resolve this contradiction and/or remove statement suggesting a dose response because of a lack of statistical significance between treatments.	S
18	1.1.5	1-48	Lines 13-15: “However, in pigs the N-nitroso metabolites have only been identified in trace amounts.” No reference is provided for this statement.	Please cite this reference. Major MA, Reddy G, Berge MA, Patzer SS, Li AC, Gohdes M. Metabolite profiling of [14C]hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. J Toxicol Environ Health A. 2007 Jul;70(14):1191-202	E
19	1.1.6 and 2.1.1	1-49 and 2-1 to 2-3	EPA justifies the use of a benchmark response (BMR) of 1% for seizures/convulsions based on the assertion that these effects are severe because they precede mortality (see other comments); however, the *actual* mortality levels/survival times from the studies were not evaluated as an endpoint on their own, or in conjunction with a discussion of the incidence of seizures/convulsions. In fact, often seizure was observed in animals that did not die subsequently.	Consider the mortality/lethality endpoint explicitly in the noncancer endpoints and incidence tables within the Toxicological Review, either as a subsection within “Other Toxicological Effects” or as a separate subsection. For example, consider Levine et al. 1983 as a key chronic study that evaluated this endpoint. Additionally, consider explicitly evaluating the relationship between seizures and mortality, which DoD feels is necessary to justify not using the standard BMR of 10% as the point of departure for seizure data.	S/M
20	1.2	1-69	Line 3-4. The statement “Although the MOA is unknown…” been established and is sufficient to explain the wide prevalence of the primary effect across species. The	Revise the interpretation of mechanism vs mode of action, specifically with regard to neurological endpoints.	S

			problem seems to be arising from the EPA's attempts to globalize the GABAα inhibition the brain to the urogenital and kidney effects; without full consideration of the fact that little is understood about GABAα receptor function in these other organs.		
21	1.2.1	1-69	Although it is discussed elsewhere, there are few data to support the EPA's contention that suppurative prostatitis is a marker for RDX urogenital effects. The available evidence in humans and animals consistently demonstrate a neurotoxic mode of action, not a urogenital one. The extension of a GABA influence in the urogenital tract is less supported with a MOA, yet the neurotoxic mechanism for seizure development is discounted.	Acknowledge that the available evidence less supports a RDX-mediated urogenital MOA and does support a mechanism for a neurological seizure event.	S
22	1.2.2. Carcinogenicity	1-70	DoD notes that in several bioassays there was never a statistically significant increase in lung tumors and that the authors of these studies considered the tumors to be random; therefore, the trend test applied by EPA would be more appropriate if either the carcinomas alone or carcinomas and adenomas were increased over background in at least one species or sex. Moreover, in one study and in one sex in another study, EPA could not find a trend. "Trend" findings are often suspect in their ability to draw useful conclusions.	DoD suggests that the multiple studies that could not find any statistically significant increase in tumors, even when malignant and benign tumors were combined, should be strong evidence of a lack of carcinogenicity for RDX. DoD requests that EPA reconsider the carcinogenicity conclusions.	S/M
23	1.2.3	1-71 to 1-72	The presence of RDX in the fetus of an exposed dam or in the milk of an exposed dam is only indicative of potential exposure of the fetus and neonate, not susceptibility. Biological susceptibility refers to the	We suggest EPA remove statements that pertain only to exposure, not inherent biological susceptibility, as exposure is not the subject of the Toxicological Review.	S/M

			greater *response* to an exposure (toxicodynamics), not simply greater exposure.		
24	2.1.1 and 2.1.2	2-1 to 2-2 and 2-6	EPA notes that the dose-response relationship for neurological effects in the chronic studies was more consistent than in the subchronic studies, yet it does not consider any chronic studies as the basis for the candidate reference dose based on neurotoxicity. EPA did not select nervous system effects in Levine et al. (1983) as a possible basis for a reference dose. One of the stated reasons is a lack of incidence data. As stated in the preamble “If a point of departure cannot be derived by modeling, a no-observed-adverse-effect level is used instead” (p. xxvii, line 48). Thus, a lack of incidence data is not necessarily a criterion for exclusion of the study. EPA further identifies uncertainty associated with the identification of the NOAEL from Levine (1983). However, we believe these concerns are overstated; the reported clinical observations were quite specific, including timing of emergence of different effects (e.g., hyperactivity vs. convulsions).	The NOAEL for neurological effects in the chronic rat study of Levine et al. (1983) should be considered as a point of departure. Uncertainties in NOAEL identification could be addressed later, qualitatively and through uncertainty factors, if appropriate.	S/M
25	2.1.2	2-4	EPA used a study with 20 animals per dose level (10 males and 10 females) to estimate a BMR of 1%. EPA justified this choice by concerns about “severity” and association with mortality. (1) Such an action is not supportable on statistical grounds and is contrary to EPA BMD guidance. (2) Mortality was assessed directly in numerous studies so it is not necessary or appropriate to use seizures as a surrogate endpoint	We suggest that EPA use a BMR of 10% for seizures, and assess mortality separately. (see other related comments)	S/M

			for mortality. See the EPA BMD guidance on p. xxv, lines77-91 and p. xxvi, lines 1-14.		
26	2.1.2	2-6	We agree with the EPA's statements that seizures were more strongly correlated with dose than with duration of exposure (p. 1-68). Therefore, we find it surprising that EPA used area under the curve (AUC) rather than peak RDX concentration for interspecies extrapolation, since peak plasma and brain RDX concentration have been consistently associated with seizure induction. The resulting POD was converted to a BMDL01-HED using a PBPK model based on modeled arterial blood concentration. The concentration was derived from the AUC of modeled RDX concentration in arterial blood, which reflects the average blood RDX concentration for the exposure duration normalized to 24 hours.	Consider revising the assessment using peak blood (or brain) RDX concentration as the metric from which to derive the Human Equivalent Dose. DoD feels that it inappropriate to use AUC for deriving the HED for the noncancer, neurologic adverse event (i.e. seizure). The POD was derived from the Crouse study where RDX was administered daily by oral gavage. A threshold brain level of RDX is required to induce seizure, i.e. the Cmax of an administered dose. Cmax is achieved rapidly after an acute oral gavage dose (Williams 2012). Therefore, it is most appropriate to derive the HED using Cmax, not AUC. Much higher doses of RDX are required to induce seizures when administered in the feed. Furthermore, this accurately represents the real world human exposure regime.	S/M
27	2.1.2	2-7	Preamble, Section 7.4. pg xxvi, line 34-37 states that linear extrapolation is used for "Agents or their metabolites for which human exposures or body burdens are near doses associated with key events leading to an effect". The work of Williams et al. have shown that the key event (binding to GABAA receptor) is a firm finding. The key event (binding to GABAA receptor) requires very high doses of RDX in the brain, which in turn requires consistently high doses of RDX	Consistent with the IRIS Preamble, Section 7.4 Extrapolating to lower doses and response levels, reconsider the real world exposure levels of RDX as they would relate to seizures. Consider adding a synthesis discussion of the possible MOA, threshold for seizures, real-world exposures, and implications on the methods for dose-response analysis and derivation of RfD.	S

			in the blood. To extrapolate what is a very clear threshold effect (seizures) to doses that are unrealistically low (much lower than the threshold) given that RDX is not known to accumulate in the body or brain, is not consistent with the statement in the preamble. Known environmental exposures (drinking water) are already so low that RDX could never accumulate in the body.		
28	2.1.3	2-7	The text does not provide evidence why the mouse PBPK model was discounted, other than there were “major uncertainties”. More justification is needed.	Provide more justification or consider using the mouse PBPK model. It was peer reviewed and published.	S
29	2.1.3	2-7	EPA chose to use a default value for the human uncertainty factor (UFH). EPA should discuss why intraspecies human variability in their selected internal dose metric (blood AUC) cannot be addressed via PBPK modeling.	Consider using PBPK modeling to replace the toxicokinetics portion of UFH with a data-derived extrapolation factor. At a minimum, discuss why PBPK modeling is unable to inform UFH instead of using default values. DoD feels that the use of default values should specifically be justified by explaining why the existing data do not allow a departure from default values. A charge question addressing this issue is also provided.	S
30	2.1.3	2-8	If AUC (rather than peak concentration) of RDX is the internal dose most relevant to risk, the timing of the FOB tests conducted by Crouse et al. (2006) should be of minimal concern. It appears that this is an inconsistency within the decision logic of the document. EPA should be consistent and either accept the validity of the FOB tests, or use peak blood	DoD feels that there is an inconsistency within the document wherein EPA relied on AUC for the internal dose metric, but then notes (and uses as justification for the UFD) the study author’s concerns regarding the timing of FOB tests (Crouse et al. (2006)), which would be a more applicable concern for peak blood concentration as the dose metric.	S

			concentration as the basis for interspecies extrapolation.		
31	2.1.3	2-8	DoD feels that the UFD of 3 is not supported. Line 31 states “Given the reports of neurobehavioral effects in several studies, additional systematic evaluation of neurobehavioral effects would be informative.” DoD agrees that additional studies might be informative; this alone does not support a UFD of 3. EPA does not provide evidence to suggest that fetal or neonatal animals have greater susceptibility to the neurotoxic (or any other) effects of RDX; exposure does not equal susceptibility. No selective reproductive/developmental toxicity of RDX was noted in the two-generation rat study (Cholakis et al., 1980), and there are other neurobehavioral evaluations conducted in several publications. DoD notes that the RDX RfD was initially published in 1988 and revised in 1993. It was based on information from a chronic rat study with a composite UF of 100 - 10 for inter and 10 for intra species variation. The 1988/93 RfD did not include a UF for inadequate database and in fact, the IRIS section I.A.5 listed overall confidence in the RfD as high and this included high confidence in the database. The current RDX database includes human data from accidental ingestion, occupational exposure information and an extensive list of publications and reports from controlled animal exposures. There are acute and sub-chronic studies in multiple species, developmental studies, mutagenicity test batteries and carcinogenicity studies. Since publication of the original RfD, there are additional data from animal	Consider eliminating the database uncertainty factor (i.e., set UFD = 1). As currently written, the UFD of 3 is not well justified (exposure does not indicate susceptibility and sufficient developmental, including neurodevelopmental studies have been conducted.) The database includes all required studies, per EPA guidance on evaluation of the database uncertainty factor. DoD has also provided a charge question to address this concern.	S/M

			toxicity studies as well as uptake metabolism and excretion studies, mechanistic studies and extensive PBPK modeling work. Moreover, the USEPA cites UFs are used when 2-generation and reproductive data are not available, however, both are available in the case of RDX. Despite the extensive initial database and the additional information accrued over the past 20 years, the latest derivation of the RfD includes a UF for data inadequacies. This seems inconsistent and difficult to defend.		
32	2.1.5	2-12	Purity, particle size and formulation was discussed as factors potentially contributing in the variation of results between studies, however, no mention is made regarding differences in bolus and feeding methods of administration (i.e. importance of ADME in the observation of effects).	As previously discussed in DoD comments, we recommend that EPA reevaluate the data and consider marked differences in response between feeding and gavage studies. Kinetics differences will be useful for explaining the variation in results between study designs.	S/M
33	2.3.1	2-15	DoD feels that the statistical evidence and biological plausibility do not support a derivation of an oral slope factor for RDX. See detailed comment on the corresponding Executive Summary discussion. Furthermore, the use of Lish et al. (1984) disqualifies an important requirement of the “suggestive evidence” well-conducted study, quantitative analyses may be useful for some purposes”. DoD submits that exceeding the Maximum Tolerated Dose of 175 mg/kg-d that caused mortality and then reducing the dose to 100 mg/kg-d would preclude the study’s use in quantifying an oral slope factor. Rather than derive an authoritative consensus value for the oral slope factor,	Given the lack of a weight of evidence for carcinogenicity, and the poor study quality of Lish et al. (1984), consider that the uncertainty is too great to quantify a slope factor for risk assessment purposes. As noted on the charge questions document, please consider at least including a charge question as to whether an oral slope factor should be derived at all, or derived as an appendix value, consistent with previous EPA decisions for which the evidence of carcinogenicity is “suggestive.” http://www.epa.gov/iris/subst/1023.htm	S/M

			particularly when these values are often then used by other regulatory authorities for purposes beyond what the EPA intends, EPA could provide an estimate as an appendix, as was done for tetrahydrofuran (U.S. EPA, 2012).		
34	2.3.2	2-17	line 22 - the HED obtained from the model-estimated amount of total RDX metabolites scaled by BWÃ‚Â¾ was equal to that calculated using administered dose scaled by BW3/4. Sweeney did a careful mouse PK study of RDX kinetics by the oral route. The USEPA decided not to utilize this work and used the default body weight extrapolation to derive a HED, despite the fact that they use PBPK modeling for non-cancer.	DoD requests a better justification supporting the use of default approaches when data are available. In addition, DoD recommends that EPA clarify why they dismiss the argument for using non-linear extrapolation for derivation of a cancer RfD. DoD suggests that EPA discuss the option of derivation of a cancer RfD and provide better scientific justification for defaulting to derivation of an OCSF.	S/M
35	Literature Search	LS 10. Line 15-16	States that “Hart (1976) used a dose range that was lower than the subsequent studies (high dose 10 mg/kd-day) and that may not have been sufficient to elicit some effects in treated animals”. While this study had many difficulties, the fact that no seizures were observed at that dose would indicate somewhat of a threshold for a chronic study.	Consider evidence that supports a threshold for seizures in vertebrates, including humans.	S
36	Literature Search.	LS-4	DoD disagrees with the classification of one study: the technical report by Bannon (2006) entitled “Biomarkers of RDX in Breath of Swine” is listed as a Secondary Source of information under “References Added During Assessment Development”. However, this study tracked the concentration of RDX in blood of juvenile pigs after a single oral dose of pure RDX in a gel capsule, which better represents exposure than	Reconsider using the Bannon 2006 report as a secondary reference source in support of RDX levels that cause seizures in mammals. Add the reference to the text of the document.	S

			the RDX dissolved in a mixture of methycelluose and tween in the rat studies. While animals seized at lower than expected doses (10 mg/kg), no mortalities were recorded and the blood RDX level reported at the time of seizure was remarkably similar to that found in rats (Williams et al, 2009) dosed with 75 mg/kd RDX. This shows that there is a consistently critical internal threshold level for seizures across species. This study should receive a second review and should be included in “Supporting Animal Studies” under “Acute Short Term Studies”.		
37	References	NA	An important reference is missing from the references and database. "Development of a Relative Source Contribution Factor for Drinking Water Criteria: The Case of Hexahydro-1,3,5-trinitro-1,3,5-triazien (RDX)."	Please add this reference. Bernard Gadagbui, Jacqueline Patterson, Andrew Rak, Raymond S. Kutzman, Gunda Reddy, and Mark S. Johnson. Development of a Relative Source Contribution Factor for Drinking Water Criteria: The Case of Hexahydro-1,3,5-trinitro-1,3,5-triazien (RDX). Human and Ecological Risk Assessment. 18; 338-354. 2012.	S
38	References	R-3	Refererence under Musick et al (2010) requires a correct report number.	Please add the number ADA526472 to the reference for Musick et al.	E