UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C. 20460

OFFICE OF THE ADMINISTRATOR
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September 27, 2017

EPA-SAB-17-011

The Honorable E. Scott Pruitt
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, D.C. 20460

Subject: Review of EPA's Draft Assessment entitled *Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (September 2016)*

Dear Administrator Pruitt:

The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review the draft assessment, entitled *Draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).* The draft assessment is based on a review of available scientific literature on the toxicity of RDX. The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of RDX-induced cancer and noncancer health effects. In response to EPA's request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with subject matter experts to conduct the review.

The SAB finds the draft assessment to be comprehensive and generally well-written. The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly conveys the major findings.

The draft assessment evaluates and modifies available physiologically-based pharmacokinetic (PBPK) models in the literature. The SAB finds the revised rat and human PBPK models to be a distinct improvement over the original approach, and these changes adequately represent RDX toxicokinetics. The application of revised PBPK models in the assessment to the calculation of human equivalent doses (HEDs) for the points of departure (PODs) for neurotoxicity and other noncancer endpoints is scientifically supported. For the hazard identification and dose-response assessment of noncancer endpoints, the SAB agrees that neurotoxicity, including seizures or convulsions, is a human hazard of RDX exposure, and supports the selection of convulsions as the endpoint for dose-response assessment. However, convulsions in rodents only provide a limited spectrum of the potential human hazard, since convulsive or non-convulsive seizures, epileptiform discharges, reduction in seizure threshold, subchronic sensitization, and neuronal damage can all be part of the spectrum of RDX's nervous system hazards. Thus, further explanation

should be provided in the draft assessment for these potential endpoints. The SAB agrees that RDX-induced convulsions arise primarily through a mode of action involving RDX-induced blockade of the gamma-amino butyric acid type A ($GABA_A$) receptor ($GABA_AR$). The SAB also agrees with the characterization of convulsions as a severe endpoint, and concludes that its potential relationship to mortality is clearly described. However, the SAB recommends that EPA revisit the benchmark response (BMR) evaluation, and at a minimum, provide a more thorough justification for using a BMR of 1% for deriving the lower bound on the benchmark dose (BMDL) as the point of departure (POD) from Crouse et al. (2006). Given that a BMR of 1% corresponds to a response that is a factor of 15 below the lowest observed response data, the SAB considers the use of BMR of 5% based on the Crouse study to be more consistent with the observed response at the Lowest-Observed-Adverse-Effect-Level (LOAEL) of 15%, and not so far below the observable data. Thus, the SAB recommends EPA to consider use of a 5% BMR while addressing the uncertainty of a frank effect with the application of uncertainty factors, or as noted above provide a more thorough justification for its choice of a 1% BMR.

With respect to the application of uncertainty factors (UF) to the PODs, the SAB supports the application of an interspecies UF of 3 to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolation from animals to humans that is not accounted for by the toxicokinetic modeling. In addition, the SAB agrees with the LOAEL to No-Observed-Adverse-Effect-Level (NOAEL) UF of 1, and the UF of 10 to account for intra-human variability. However, the SAB has concerns about the use of a subchronic to chronic UF (UF_S) of 1. An i*n vitro* assessment of GABA activity has shown that the effects of RDX are not reversible following compound wash out (Williams et al. 2011), making it possible that repeated exposures to RDX have cumulative effects on GABAergic neurotransmission. Thus, the SAB recommends that EPA reconsider the UF for subchronic to chronic extrapolation, and that at a minimum, provide a stronger justification for a UF_S of 1. Further, the SAB disagrees with the application of a database uncertainty factor (UF_D) of 3, and recommends EPA apply an UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe nervous system effects, and proximity of the dose that induces convulsions with the dose that induces mortality. In total, a composite UF of 300 should be considered instead of the UF of 100, as proposed in the draft assessment.

The SAB supports the derivation of a reference dose (RfD) for nervous system effects, but finds the scientific rationale for the proposed RfD to be incomplete due to concerns regarding the choice of the BMR and the choice of value for uncertainty factors. While the SAB supports the use of the dose-response data from the Crouse et al. (2006) study in the assessment as the primary basis for the derivation of an RfD for neurotoxicity, EPA should more fully account for database uncertainty, as a POD based on convulsions does not capture all of the potential adverse outcomes, or their severity. Sufficiently sensitive test batteries to detect neurobehavioral consequences produced by chronic/subchronic exposure to RDX, especially during pregnancy, have not been conducted. Moreover, tests designed to detect subtle developmental neurotoxic effects during the perinatal-weaning period have also not been conducted. These concerns are especially compelling because of more recent peer-reviewed published data indicating that subconvulsive doses of either bicuculline (which has a similar mechanism of action to RDX) or domoic acid

(which has agonist activity on glutamate transmission) cause developmental and behavioral impairments at doses below those that cause convulsions. Thus, the significant data gap on the lack of developmental neurotoxicity study of RDX needs to be considered.
The SAB agrees that kidney and other urogenital system toxicity are a potential human hazard of RDX exposure. However, the SAB disagrees with the selection of suppurative prostatitis as the "surrogate marker" to represent this hazard, and recommends that EPA considers suppurative prostatitis a separate effect. As such, separate organ/system-specific RfDs should be derived for the kidney and urogenital system, based on findings of renal papillary necrosis and associated renal inflammation, and for suppurative prostatitis, respectively.

The SAB disagrees with the conclusion that male reproductive effects are a human hazard associated with RDX exposure as the database does not support this conclusion, and concludes that the proposed RfD for reproductive system effects in the draft assessment is not scientifically supported. Moreover, the SAB concludes that RDX does not pose a risk of induction of structural malformations during human fetal development based on animal data. The SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity, which were only seen at maternally toxic dose levels. Lastly, the SAB also notes that potential neurodevelopmental toxicity based on the reported mechanism of RDX inhibition of GABAergic neurons, and the findings that RDX is present in the brains of offspring rats and in the milk from dams treated with RDX during gestation, were not adequately discussed in the draft assessment.

With regard to dose-response analysis of noncancer effects, the SAB agrees that the overall RfD should be based on nervous system effects. The SAB agrees with the use of the dose-response data from the Crouse et al. (2006) study as the primary basis for the derivation of an RfD and recommends that EPA strengthen the justification for not using the dose-response data from Cholakis et al. (1980) for RfD derivation.

With regard to cancer effects, the SAB agrees that "suggestive evidence of carcinogenic potential" is the most appropriate cancer hazard descriptor for RDX, in accordance with EPA's *Guidelines for Carcinogen Risk Assessment*; and that this descriptor applies to all routes of exposure. The SAB also agrees with the EPA's rationale for a quantitative cancer dose-response analysis for RDX and the use of the linear low-dose extrapolation approach, since the mode of action for cancer is unknown. However, the SAB finds that the calculations of the PODs and oral slope factor (OSF) were not clearly described. The SAB recognizes the EPA's preference for using the multistage model for cancer dose-response modeling. However, the SAB has identified a number of concerns with the data used to derive the cancer POD, the rationale for restricting modeling to the multistage model to derive the POD, and the conditions under which the EPA's MS-COMBO multi-tumor modeling methodology provides a valid POD and cancer slope factor estimate. The SAB makes multiple recommendations on how the discussion on the derivation of the OSF can be improved.

The SAB appreciates this opportunity to review EPA's *Draft Toxicological Review of RDX* and looks forward to the EPA's response to these recommendations.

Sincerely,

/s/

Dr. Peter S. Thorne
Chair
Science Advisory Board

/s/

Dr. Kenneth S. Ramos
Chair
SAB CAAC Augmented for the Review of the Draft IRIS RDX Assessment

Enclosure

NOTICE

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ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criteria
AST	Aspartate Aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area Under the Curve
BDNF	Brain-Derived Neurotrophic Factor
BLA	Basolateral Amygdala
BMC	Benchmark Concentration
BMCL	Lower 95% Confidence Limit of the Benchmark Concentration
BMD	Benchmark Dose
BMDL	Lower 95% Confidence Limit of the Benchmark Dose
BMR	Benchmark Response
BW	Body Weight
CAAC	Chemical Assessment Advisory Committee
CI	Confidence Interval
CPK	Creatine Phosphokinase
EPA	Environmental Protection Agency
GABA	Gamma-Amino Butyric Acid
$GABA_AR$	Gamma-Amino Butyric Acid Type A Receptor
GABAergic	Pertaining to or affecting Gamma-Amino Butyric Acid
HED	Human Equivalent Dose
HERO	Health and Environmental Research Online
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
IARC	International Agency for Research on Cancer
ILSI	International Life Sciences Institute
IPSPs	Inhibitory Postsynaptic Potentials
IRIS	Integrated Risk Information System
IUR	Inhalation Unit Risk
K_i	Inhibition Constant
LD	Lethal Dose
LOAEL	Lowest-Observed-Adverse-Effect Level
miRNA	MicroRNA
MEDINA	Methylenedinitramine
MNX	Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
MOA	Mode of Action
NAS	National Academy of Sciences
NCI	National Cancer Institute
NDAB	4-Nitro-2,4-diazabutanal
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
ORD	Office of Research and Development

OSF	Oral Slope Factor
PBPK	Physiologically Based Pharmacokinetic
PND	Postnatal Day
POD	Point of Departure
PTX	Picrotoxin
PWG	Pathology Working Group
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RfC	Reference Concentration
RfD	Reference Dose
ROS	Reactive Oxygen Species
RR	Relative Risk
SAB	Science Advisory Board
SDMS	Spontaneous Death or Moribund Sacrifice
TNX	Hexahydro-1,3,5-trinitroso-1,3,5-triazine
UCL	Upper Confidence Limit
UF	Uncertainty Factor
UF_D	Database uncertainty factor
UF_H	Human Inter-individual Variability Uncertainty Factor
UF_L	LOAEL-to-NOAEL Uncertainty Factor
UF_S	Subchronic-to-chronic Uncertainty Factor
WHO	World Health Organization

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA's Integrated Risk Information System (IRIS) program to review the EPA's *Draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (September 2016)* (hereafter referred to as the draft assessment). EPA's IRIS is a program that evaluates information on human health effects that may result from exposure to environmental contaminants. The draft assessment consists of a review of the available toxicological scientific literature on RDX. The draft assessment was revised in September 2016 and a summary of EPA's disposition of the public comments received on an earlier draft version of the assessment was added to the Toxicological Review in Appendix E of the Supplemental Information.

Literature Search Strategy/Study Selection and Evaluation

In general, the literature search strategy, study selection considerations, and study evaluation considerations, including inclusion and exclusion criteria, are well-described, documented, and appropriate. However, the SAB identified several areas that EPA's literature search missed and that should have been covered, including literature on the role of GABAergic systems in brain development and the potential developmental neurotoxicity of RDX through interference with GABAergic systems. In addition, EPA should clarify in the literature search strategy section its reasoning and approach for including or excluding studies on nonmammalian species along with secondary references. The SAB notes that the metabolism of RDX has not been adequately studied, and suggests that the lack of toxicological data for the anaerobic bacteria metabolite, methylenedinitramine (MEDINA) and mammalian oxidative transformation product 4-nitro-2,4-diazabutanal (NDAB), and 4-nitro-2,4-diazabutanamide be noted in the assessment. The SAB identified additional peer-reviewed studies from the literature, which the EPA should consider in the draft assessment.

The sections below provide details of the evaluation and conclusions reached by the SAB. Key and suggested recommendations for the revision of the draft assessment are provided in response to the charge questions. Key recommendations are those the SAB deemed essential for inclusion in the assessment, while suggested recommendations are offered as options for consideration by the EPA. In addition, per EPA's request, future research needs are provided in Section 4 of this report.

Toxicokinetic Modeling

The SAB finds the conclusions reached by the EPA following its evaluation of the PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b) to be well-documented and scientifically supported. The modifications that the EPA made to the PBPK models of Krishnan/Sweeney represent distinct improvements over the original approach, and these changes adequately represent RDX toxicokinetics. The EPA also performed validation of the PBPK model using independent rat data sets, and all models provided reasonable fits according to standard goodness-of-fit measures. The SAB finds the uncertainties in the model to have been well described.

The SAB concludes that the choice of dose metric for neurotoxicity is clearly described. Without brain RDX concentration data, plasma or blood concentration data are used as a surrogate for brain concentrations. The EPA's approach is adequately justified, since limited pharmacokinetic data in mice, rats, swine and humans show concordance between blood and brain RDX levels over time following exposure. The use of area under the curve (AUC) in a plasma concentration-time plot as a dose metric for interspecies extrapolation to humans from oral points of departure (PODs) derived from rat data is justified. AUC is representative of the average RDX plasma concentration over a dosing interval, i.e., 24-hour interval. Published blood and brain RDX levels in rats for 24-hour time-courses appear to coincide with symptomatology. The mouse model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model as well as uncertainties associated with selection of a dose metric for cancer endpoints. This decision is scientifically supported and clearly explained.

Hazard Identification and Dose-Response Assessment

Nervous System Effects

The available human, animal, and mechanistic studies support EPA's conclusions that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. Furthermore, RDX-induced convulsions arise primarily through a rapid mode of action resulting from RDX-induced blockade of the $GABA_A$ receptor ($GABA_AR$) (Williams et al. 2011). Despite the limitations of the only cross-sectional study of Ma and Li (1993), which indicated significant neurobehavioral and memory deficits associated with RDX exposure for 60 workers in a Chinese RDX plant, there is sufficient evidence from clinical case reports, animals and mechanistic studies of RDX to support EPA's conclusion that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. However, the SAB concludes that the evidence presented in the draft assessment does not adequately depict RDX's hazards to the nervous system because convulsions in rodents only provide a limited spectrum of potential human hazard. In this regard, convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, subchronic sensitization, and neuronal damage can all be part of the spectrum of RDX's nervous system hazards. Moreover, tests directed at detecting subtle developmental neurotoxicity during the perinatal-weaning period have not been conducted. These concerns are especially compelling because of more recent peer-reviewed published data indicating that sub-convulsive doses of either bicuculline (which has a similar mechanism of action to RDX) or domoic acid (which has agonist activity on glutamate transmission) cause developmental and behavioral impairments at doses below those that cause convulsions. Therefore, there are data gaps among existing studies to address the complete spectrum of RDX effects. Future studies addressing cognitive and behavioral effects, as well as developmental neurotoxicity of RDX would assist in assessing other endpoints less severe than convulsions

The SAB finds the selection of studies reporting nervous system effects to be scientifically supported and clearly described; although quality issues in the Cholakis et al. (1980) study as detailed below should be more fully described. Further, the SAB concludes that it is appropriate to consider the dose-response data reported in Crouse et al. (2006) as a relevant model. While this study utilized gavage administration of RDX rather than a dietary route of administration (which most likely represents the route of exposure for the general population), there is considerably less variability in the amount of the toxic agent delivered by gavage compared to dietary intake and

gavage is independent of feeding patterns. The SAB recognizes that the use of a gavage study rather than a dietary study allows for some unquantified margin of safety in the derived RfD. The SAB agrees that the characterization of convulsions as a severe endpoint, and its potential relationship to mortality, are appropriately described.

The SAB finds that the selection of convulsions as the endpoint to represent nervous system hazard for RDX is scientifically supported and clearly described. Convulsion is the most biologically significant endpoint that has been reasonably and reliably measured for RDX. However, evidence from studies of other seizurogenic compounds with a mode of action similar to RDX suggests that there are other, generally subclinical, cognitive and behavioral neurological effects that occur at doses below those causing seizure activity. The SAB agrees that the likely dose range between convulsion and other nervous system effects can be addressed using UF adjustments. The SAB also finds that the calculation of the HEDs using PBPK modeling for the convulsion studies in rats to be scientifically supported and clearly described, and endorses the approach of estimating the effective concentration as the area under the curve (AUC) of concentration and time.

The SAB identified several concerns regarding EPA's use of a BMR of 1% for benchmark dose modeling of the Crouse et al. (2006) data for convulsions. EPA's choice of a BMR of 1% for modeling is based on the severity of the convulsion endpoint and the proximity of doses that cause convulsions to lethality. In the Crouse study, a BMR of 1% would correspond to a response that is a factor of 15 below the lowest observed response data. The SAB agrees that both the severity of convulsions as an endpoint and the proximity of convulsive doses to lethal doses are valid sources of uncertainty in terms of providing sufficient protection for sensitive human populations. However, the SAB concludes that uncertainty about the appropriateness of the dose-response data and the POD derived from those data should be addressed through UFs and not through unsupported extrapolation of the dose-response data. As indicated in the EPA guidance document, the greater the "distance" between the observable data and the BMD, the greater the statistical uncertainty in the fit of the model at the BMD, and therefore, the greater the difference between the BMD and the BMDL. A BMR of 5% based on the Crouse study is more consistent with the observed response at the Lowest-Observed-Adverse-Effect-Level (LOAEL) of 15% and not so far below the observable data. On this basis, EPA should consider use of a 5% BMR with additional uncertainty factor to address the concern over using convulsions as the toxicological endpoint for the RfD. At a minimum, EPA should provide a more thorough justification for its choice of a 1% BMR, and specifically justify why a 1% BMR is a more appropriate extrapolation than a 5% BMR, and why the greater conservatism in risk assessment required for a frank effect is better dealt with through a lower BMR than through application of UFs.

With respect to the application of UFs to the PODs, the SAB supports the application of an interspecies UF of 3 to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolation from animal to human that is not accounted for by the toxicokinetic modeling, a LOAEL to No-Observed-Adverse-Effect (NOAEL) UF of 1, and an UF of 10 for intra-human variability. However, the SAB has concerns about the use of a subchronic to chronic UF (UF_S) of 1. Data generated using an *in vitro* assay for GABA activity show that the effects of RDX were not reversible following compound wash out (Williams et al. 2011). As such, repeated exposures to RDX may have cumulative effects on GABAergic neurotransmission. The SAB recommends

that EPA reconsider the UF for subchronic to chronic extrapolation, and at a minimum, provide stronger justification for the use of a UF_S of 1. Further, the SAB disagrees with the application of a database uncertainty factor (UF_D) of 3, and recommends EPA consider applying a UF_D of 10 to account for data gaps in developmental neurotoxicity, lack of incidence data for less severe effects, and proximity of the dose inducing convulsions to that inducing mortality. In sum, a composite UF of 300 should be considered instead of 100 as proposed in the draft assessment.

The SAB finds the scientific support for the RfD derived by EPA for nervous system effects to be incomplete for the reasons outlined above, and concludes that a POD based on convulsions does not capture all of the potential adverse outcomes, or their severity. While the SAB supports the use of the dose-response data from the Crouse et al. (2006) study as the primary basis for the derivation of an RfD for neurotoxicity, EPA should more fully account for database uncertainty.

Kidney and other Urogenital System Effects

The SAB agrees that the available human, animal, and mechanistic studies support the conclusion that kidney and other urogenital system toxicities are a potential human hazard of RDX exposure. However, this conclusion is primarily supported by animal data, given that available human studies identifying the kidney as a potential target of RDX are sparse and only identify transient renal effects following acute human exposure. There are no reports of prostatic effects of RDX in humans and no pertinent mechanistic data regarding RDX effects on the kidney and urogenital system. The SAB finds all hazards to the kidney and urogenital system adequately assessed and described in the draft assessment, with the exception of the description of inflammatory changes in the rat prostate. The SAB concludes that the selection of suppurative prostatitis as the endpoint to represent this hazard was clearly described in the draft assessment, but not scientifically supported because no known mechanistic link exists between suppurative prostatitis and renal papillary necrosis or adverse effects in the kidney.

The SAB finds that the selection of the Levine et al. (1983) study to evaluate kidney and other urogenital system effects was clearly described, but not entirely supported by scientific evidence. Mild toxic effects of RDX exposure on the kidney were found in some species, but not others. In some studies, toxic effects were seen in both sexes, while in others only male or female effects were observed. Of note is that some of these effects (i.e., mineralization) occurred in a small study with non-human primates, while some rodent studies did not find evidence of renal toxicity. Only the chronic study of Levine et al. (1983) showed severe toxic effects on the kidney, and this was only seen in males at the highest dose (40 mg/kg-day); bladder toxicity also occurred in this treatment group, whereas effects on the prostate occurred at doses of 1.5 mg/kg-day and above. Therefore, the SAB determines that the selection of suppurative inflammation of the prostate as a "surrogate marker" of the observed renal and urogenital system effects for derivation of a reference dose is not justified. The SAB recommends that a separate RfD be derived for the kidney and urogenital system based on renal papillary necrosis and associated renal inflammation and that the male accessory sex glands be designated as a separate organ system, with a separate RfD derived based on suppurative prostatitis.

As for the calculation of the POD and HED for suppurative prostatitis as a stand-alone endpoint, both are scientifically supported and clearly described. The application of UFs should be the

same as those for nervous system effects, if this system-specific RfD is to be considered for selection as an overall RfD.

Developmental and Reproductive System Effects

The SAB disagrees with the conclusion in the draft assessment that there is suggestive evidence of male reproductive effects associated with RDX exposure. The available animal evidence based on testicular degeneration in male mice exposed to RDX in their diet for 24 months (Lish et al. 1984) is weak, unsupported by other endpoints in that study, complicated by the age of the mice and the general toxicity of the RDX dose used, and contradicted by most other studies. Thus, the database as a whole does not support this conclusion. There is no human evidence indicating male reproductive toxicity; no human studies have focused on this question, and there were no incidental reports of reproductive effects following RDX exposures. The SAB also finds adequate evidence from animal studies to conclude that RDX does not pose a risk of induction of structural malformations during human fetal development based on studies on rats and rabbits at doses that were high enough to occasionally produce maternal toxicity. Additionally, the SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity, which only occurred at maternally toxic dose levels. Further, he SAB concludes that RDX presents a potential neurodevelopmental hazard that was not adequately addressed in the draft assessment. A pilot developmental neurotoxicity study in rats found a significant concentration of RDX in the immature brain of offspring and in milk from dams treated with 6 mg/kg-day of RDX during gestation. Given that Lish et al. (1984) was used for the calculation of a POD and HED for the derivation of an organ/system-specific reference dose for reproductive system effects, the RfD based on testicular degeneration is not scientifically supported.

Other Noncancer Hazards

The SAB considers it important that the draft assessment be explicit as to whether the available evidence does or does not support liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects as a potential human hazard, and the rationale for reaching that conclusion. In addition, body weight gain should be included in this evaluation as it has been identified as a potential adverse effect of RDX exposure elsewhere (Sweeney et al. 2012a, b).

Cancer

The SAB concurs with the EPA that "*suggestive evidence of carcinogenic potential*" is the most appropriate cancer hazard descriptor for RDX and that this descriptor applies to all routes of human exposure. The SAB agrees with the EPA that the relevant observations are the liver tumors observed in female B6C3F1 mice and male F344 rats and lung tumors that were observed in female B6C3F1 mice in two-year dietary bioassays (Lish et al. 1984; Levine et al. 1983). The SAB identifies a number of limitations for these studies and concludes that the evidence for a positive tumor response to RDX in two species, two sexes, or two sites, required by EPA's *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005) for a "*likely to be carcinogenic to humans"* descriptor, is weak or absent. On these bases, the SAB concludes that the descriptor, "*suggestive evidence of carcinogenic potential,"* is appropriate. The SAB also finds that the draft assessment adequately explains the rationale for a quantitative cancer dose-response analysis for RDX. Lish et al. (1984) was a well-conducted two-year bioassay that included a large number of animals tested at multiple dose levels, and increased incidences of neoplasms occurred in exposed female mice. Moreover, the updated liver tumor incidences from a Pathology Working Group reanalysis

of Lish et al. (1984) were used by EPA for quantitative dose-response analysis. The study is suitable and appropriate for dose-response assessment, consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*.

With regard to the cancer dose-response assessment, the SAB supports the use of a linear low-dose extrapolation approach, as the mode of action for cancer resulting from RDX exposure is unknown. The SAB finds that the calculations of the PODs and OSF are not clearly described, and the SAB has concerns with the quality of the data used to derive the cancer POD, the rationale for restricting modeling to the multistage model, and with the conditions under which the EPA's MS-COMBO multi-tumor modeling methodology provides a valid POD and cancer slope factor estimate. The SAB also has concerns with the unexpectedly low 1.5% incidence of liver tumors in female control mice and its impact on dose-response modeling. In addition, the draft assessment relies on the multistage model to describe the POD and cancer slope factor. While understanding the preference of the IRIS program for the multistage model form, the SAB recommends that at a minimum, the draft assessment should discuss the adequacy of the fit of the multistage model to the available data. The SAB also recommends that a more detailed description of the EPA's MS-COMBO modeling methodology be provided in the draft assessment to include a description of the independence assumption and the impact of violations of this assumption on the estimated POD. Lastly, the SAB questions the inclusion of the highest dose group in dose-response modeling of liver tumors in female B6C3F1 mice.

Dose-Response Analysis

Oral Reference Dose for Effects Other Than Cancer

Although the SAB agrees that neurotoxicity should be the basis for an overall RfD for RDX, the SAB finds that the scientific support for the proposed overall RfD is incomplete, as evidenced by concerns regarding the choice of the BMR and resultant model uncertainty and choice of the values for uncertainty factors. The SAB agrees with EPA's use of the dose-response data from the Crouse et al. (2006) study as the primary basis for the derivation of the overall RfD. Table 4 provides a comparison of derived candidate RfD values using different PODs and composite uncertainty factors. The SAB makes recommendations regarding the choice of the BMR and uncertainty factors to improve the oral RfD.

Inhalation Reference Concentration for Effects other than Cancer

There are no toxicokinetic data from inhalation exposures of laboratory animals or humans to RDX. There are epidemiological studies of persons exposed occupationally to RDX, but no information was provided on exposure levels. In light of the lack of toxicokinetic data and exposure levels, an inhalation reference concentration cannot be derived.

Oral Slope Factor for Cancer

The SAB finds that the calculation of an OSF for cancer endpoints is not clearly described in the draft assessment, and has questions about whether the OSF is scientifically supported. The SAB makes multiple suggestions on how the discussion can be improved.

Inhalation Unit Risk for Cancer

There are no toxicokinetic data from inhalation studies of RDX in laboratory animals or humans, no inhalation carcinogenicity bioassays of RDX, nor data on cancer incidence in humans. Therefore, an inhalation unit risk for cancer cannot be derived.

Executive Summary

Generally, the SAB considered the Executive Summary to be well written, succinct, and clear. As changes are made to the body of the draft assessment in response to the SAB's recommendations, the Executive Summary should be updated accordingly. In addition, the SAB offers a number of specific suggestions for improving the Executive Summary.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the EPA's *Draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)* (hereafter referred to as the draft assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The draft assessment consists of a review of available scientific literature on RDX. The draft assessment was revised in September 2016 and a summary of EPA's disposition of the public comments received on an earlier version of the assessment was added in Appendix E of the Supplemental Information to the Toxicological Review.

In response to the EPA's request, the SAB convened an expert panel consisting of members of the Chemical Assessment Advisory Committee augmented with subject matter experts to conduct the review. The SAB panel held a teleconference on November 17, 2016, to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on December 12 - 14, 2016, to discuss responses to charge questions and consider public comments. The SAB panel also held teleconferences to discuss their draft report on April 13, 2017, and April 17, 2017. Oral and written public comments have been considered throughout the entire advisory process.

This report is organized to follow the order of the charge questions. The full charge to the SAB is provided as Appendix A. Editorial comments from the SAB are provided in Appendix B. The SAB also provides suggestions on the format of EPA's charge questions in Appendix C.

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. Literature Search/Study Selection and Evaluation

Charge Question 1. The section on Literature Search Strategy| Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search strategy, study selection considerations, and study evaluation considerations, including inclusion and exclusion criteria, are mostly well-described, documented, and appropriate, with a few exceptions as noted below. EPA suitably cast a wide net to retrieve all pertinent studies for the evaluation of health effects associated with RDX exposure. They searched PubMed, Toxline, Toxcenter, Toxic Substances Control Act Test Submissions (TSCATS), and the Defense Technical Information Center (DTIC) database, a central online repository of defense-related scientific and technical information within the Department of Defense. Studies were then screened to find those relevant to assessing the adverse health effects of exposure to RDX and developing a dose-response assessment. Citations in review articles and citations within original articles were also obtained and screened for additional pertinent information.

Figure LS-1 and Table LS-1 provide a summary of the general inclusion and exclusion criteria for studies that were considered for further evaluation of potential health effects of RDX. EPA used criteria to exclude studies such as citations that were abstract only, on treatment and mitigation of environmental contamination with RDX, on laboratory methods, and those on the physical-chemical properties including explosivity. These were appropriate exclusion criteria, in the SAB's opinion. These criteria resulted in the exclusion of over 900 references from further evaluation. The SAB thought that Figure LS-1 could be made clearer and better coordinated with the inclusion and exclusion criteria described in Table LS-1. Some exclusion criteria (e.g. exposure to a mixture) were included in Table LS-1, but not in Figure LS-1.

Table LS-1 indicates that studies on "ecological species" and nonmammalian species were excluded. This contradicts statements (page xxix, lines 13-16) indicating that studies on nonmammalian species and ecosystem effects were considered as sources of information for the health effects assessment. The SAB suggests that these statements be clarified, and that data for all mammalian species be retained, even if they are considered "ecological species."
The SAB notes that the exclusion of nonmammalian species may not be appropriate in light of the use of nonmammalian species such as zebrafish (e.g., in medium throughput assays for developmental neurotoxicity) to evaluate potential health risk to humans, and describe Adverse Outcome Pathways. Although there may be no studies of RDX *in vitro* or in the cellular and tissue-based high throughput assays, future research using these types of assays may provide mechanistic information for chemicals that could be used in health effects assessments.

Inclusion criteria in Table LS-1 were related to whether a citation was a source of health effects data pertinent to assessing the risk to humans (e.g., studies of health outcomes in RDX exposed humans or standard mammalian models by either the oral or inhalation route; exposure to RDX

measured; health outcomes/endpoints reported). Sources of mechanistic and toxicokinetic data were also included. Secondary references and other sources that described ecosystem effects, exposure levels, dealt with mixtures, were reviews or risk assessments and regulatory documents, were excluded from study evaluation. However, EPA indicates that secondary references containing health effects data, and citations on nonmammalian toxicity were kept for consideration in the draft assessment. The description of what was done with secondary references could be clearer and better coordinated between the text and Figure LS-1 and Table LS-1.

EPA provides details of the search in Appendix B, including search terms, and the number of hits per search term sequence per database searched. They also tabulate the number of citations added to the database from their forward and backward web of science search of specific citations. Thus, the EPA has been transparent in its process of identifying studies for evaluation.

EPA's evaluation of studies is reasonably well-described and summarized in Table LS-3. The EPA used standard criteria and questions to evaluate study quality and utility that are described in several EPA guidance documents cited in the draft assessment. Studies were evaluated considering the experimental design and conduct, issues related to exposure to RDX, endpoints evaluated, and presentation of results. EPA describes generally the issues they considered in evaluating the utility of both human and animal studies to inform both hazard identification and dose-response assessment.

EPA excluded four studies on health effects and described the reason for excluding these in Table LS-2. Similarly, EPA describes some of the important limitations in experimental animal studies in Table LS-5. Overall, the description of EPA's study evaluation is clear, although the terminology is somewhat inconsistent (e.g., methodological features in Table LS-3 do not quite match the subheadings where these are described later in the section). Some details on strengths and limitations of specific studies chosen for further evaluation are provided in subsequent sections describing hazard identification and dose-response assessment for specific organ systems.

The SAB raised concerns about an inadequate description and discussion of supporting evidence for sensitive subpopulations in the draft assessment. Although there are no adequate studies on developmental neurotoxicity of RDX, there are some mechanistic studies implicating GABA antagonist activity of RDX in the neurotoxicity observed in animals and humans. The SAB concludes it would have been appropriate to search the literature for the role of GABA in brain development to describe what is known to date and incorporate this information into the draft assessment (see additional discussion of this issue in Section 3.3.1.4). Such mechanistic information provides evidence for the existence of sensitive subpopulations (e.g., infants, children, pregnant women and their fetus), and informs the choice of UFs meant to account for variability in the human population. EPA does not discuss the role of GABAergic systems in neurodevelopment and the potential for interference with this system by RDX (or other compounds with similar molecular mechanisms) to induce developmental neurotoxicity, an omission that should be rectified. The SAB identified four references that may be used to start the discussion of the role of GABAergic systems during development and the potential for RDX developmental neurotoxicity. A listing of these references is provided below.

The SAB notes that the metabolism of RDX has not been adequately studied. Limited toxicity information for the N-nitroso metabolites of RDX, specifically hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), has been discussed in the draft assessment and in the Supplemental Information document. However, reference to the anaerobic bacteria metabolite, methylenedinitramine (MEDINA) (Fuller et al. 2009 and 2010), was not included in the metabolism section of the Supplemental Information document. N-nitroso metabolites are generated anaerobically and likely result from bacterial transformation of parent RDX in the gastrointestinal tract (Pan et al. 2007b). Although these are minor metabolites, some reductive transformation products of RDX (including MNX and TNX) are present in ground waters near munitions and training facilities (Beller and Tiemeier, 2002),

The SAB assembled five additional references to augment the neurotoxicity database. In addition, 11 references that address the production and toxicity of reductive transformation products and studies that were conducted in species that may inform the current RDX assessment are identified. A full listing of these references is provided below.

Key Recommendations:

- EPA should include a literature search on the role of GABAergic systems in brain development, and how this knowledge can inform a better understanding of the potential developmental neurotoxicity of RDX.
- EPA should not exclude nonmammalian species as they may bring important mechanistic insight into the draft assessment.
- EPA should clarify its reasoning and approach for including or excluding nonmammalian species studies and secondary references.

Suggested Recommendations

- The lack of / paucity of toxicological data for MEDINA and the mammalian oxidative transformation product 4-nitro-2,4-diazabutanal (NDAB), 4-nitro-2,4-diazabutanamide, MNX and TNX could be noted in the draft assessment.

Additional Citations for USEPA to Consider:

1. Beller, HR; Tiemeier, K. (2002). Use of liquid chromatography/tandem mass spectrometry to detect distinctive indicators of in situ RDX transformation in contaminated groundwater. *Environmental Science & Technology* 36: 2060-2066.

2. Creeley, CE. (2016) From drug-induced developmental neural apoptosis to pediatric anesthetic neurotoxicity – where are we now? *Brain Sci* 6(3):32-44.

3. Fuller, ME; Perreault, N; Hawari, J. (2010). Microaerophilic degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by three Rhodococcus strains. *Letters in Applied Microbiology* 51:313–318.

4. Fuller, ME; McClay, K; Hawari, J; Paquet, L; Malone, TE; Fox, BG; Steffan, RJ. (2009). Transformation of RDX and other energetic compounds by xenobiotic reductases XenA and XenB. *Appl Microbiol Biotechnol* 84:535-544.

5. Gust, KA; Brasfield, SM; Stanley, JK; Wilbanks, MS; Chappell, P; Perkins, EJ; Lotufo, GR; Lance, RF. (2011). Genomic investigation of year-long and multigenerational exposures of fathead minnow to the munitiont compound RDX. *Environ Toxicol Chem* 30: 1852-1864.

6. Halasz, A; Manno, D; Perreault, NN; Sabbadin, F; Bruce, NC; Hawari, J. (2012). Biodegradation of RDX Nitroso Products MNX and TNX by Cytochrome P450 XplA. *Environ Sci Technol* 46: 7245-7251.

7. Jaligama, S; Kale VM; Wilbanks, MS; Perkins, EJ; Meyer, SA. (2013). Delayed myelosuppression with acute exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and environmental degradation product hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) in rats. *Toxicol Appl Pharmacol* 266: 443-451.

8. Jeilani, YA; Duncan, KA; Newallo, DS; Thompson, AN, Jr.; Bose, NK. (2015). Tandem mass spectrometry and density functional theory of RDX fragmentation pathways: Role of ion-molecule complexes in loss of NO3 and lack of molecular ion peak. *Rapid Commun Mass Spec*t 29: 802-810.

9. Kim, JY; Liu, CY; Zhang, F; Duan, X; Wen, Z; Song, J; Feighery, E; Lu, B; Rujescu, D; St Clair, D; Christian, K; Callicot, JH; Weinberger, DR; Song, H; Ming, Gl. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148:1051-1064.

10. Marty, S; Wehrle, R; Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *The Journal of Neuroscience* 20:8087-8095.

11. Meyer, SA; Marchand, AJ; Hight, JL; Roberts, GH; Escalon, LB; Inouye, LS; MacMillan, DK. (2005). Up-and-down procedure (UDP) determinations of acute oral toxicity of nitroso degradation products of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *J Appl Toxicol* 25: 427-434.

12. Mukhi, S; Patino, R. (2008). Effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in zebrafish: General and reproductive toxicity. *Chemosphere* 72: 726-732.

13. Rivera, C; Voipio, J; Payne, JA; Ruusuvuori, E; Lahtinen, H; Lamsa, K; Pirvola, U; Saarma, M; Kaila, K. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397(6716):251-5.

14. Salari, AA; Amani, M. (2017) Neonatal blockade of GABA-A receptors alters behavioral and physiological phenotypes in adult mice. *Int J Dev Neurosci* 57:62-71.

15. Smith, JN; Pan, XP; Gentles, A; Smith, EE; Cox, SB; Cobb, GE. (2006). Reproductive effects of hexahydro-1,3,5-trinitroso-1,3,5-triazine in deer mice (Peromyscus maniculatus) during a controlled exposure study. *Environ Toxicol Chem* 25: 446-451.

16. Williams, LR; Wong, K; Stewart, A; Suciu, C; Gaikwad, S; Wu, N; DiLeo, J; Grossman, L; Cachat, J; Hart, P; Kalueff, AV. (2012). Behavioral and physiological effects of RDX on adult zebrafish. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology* 155:33-38.

3.2. Toxicokinetic Modeling

In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al. 2012a; Sweeney et al. 2012b).

3.2.1.Model Evaluation

Charge Question 2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

The conclusions reached by the EPA following its evaluation of the PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b) are well-documented and scientifically supported. EPA did a thorough and accurate job reviewing and summarizing what is known about the oral absorption of different forms/preparations of RDX, as well as the compound's distribution, metabolism and excretion. The changes that the EPA made to the PBPK model of Krishnan /Sweeney (specified on p C-15 of the Supplemental Information document) represent distinct improvements over the original approach, and these changes adequately represent RDX toxicokinetics. Human metabolic rate constants were fitted from human data. Additionally, it is stated that *in vitro* data from rats and human metabolic studies were used and scaled-up to liver size based on microsomal protein. The EPA also performed validation of the PBPK model using independent rat data sets, and the models provided reasonable fits according to standard goodness-of-fit measures. The uncertainties in the model are well-described and were appropriately considered as illustrated by the discussion of the mouse model and the decision not to implement it. Overall, the SAB finds that the model assumptions and parameters were scientifically supported and that the draft assessment does an excellent job in compiling the data presented in Appendix C.

The SAB has several suggestions based on its review of Section C.1:

- In Section C.1.2, include the tissue parent and metabolite data of Pan et al. (2013) cited elsewhere in the report.
- In Section C.1.2, provide additional text describing the distribution of RDX to the brain as a key target tissue. Issues that could be discussed in more detail include i) Brain extracellular fluid concentration-effect relationships; ii) Changes in plasma/blood concentrations over time that may be proportional to brain concentrations, and used to derive toxicity, as proposed, based on limited correlations observed with brain and plasma data from animal studies and data from a child poisoning case (Woody et al. 1986); iii) reasons leading to the decision to not use PBPK-simulated brain RDX concentrations, which were only moderately well fitted in Figure C-6, as a dosimeter for neurotoxicity risk assessment; and iv) Experimental findings lending support to the decision to use plasma as a surrogate.

- Protein binding of RDX is not mentioned in the draft assessment. This may be regarded as a potential weakness given that it is the free concentration that would diffuse across the blood-brain barrier in the absence of any active uptake processes, or be available for metabolism. Protein binding could account for differences in predicted brain/blood ratios in humans, and may be helpful in allometric scale-up of clearance. However, absent any empirical values for protein binding, the use of total, rather than free, concentrations is the only option. The SAB suggests text noting this issue could be added.

The following items could be considered by the EPA if it were to undertake a major update to the RDX PBPK model in the future.

- Despite improvements in the model, the rat data are only moderately well fitted and show substantial deviations, especially at early time-points. This may reflect deviations of the simulations due to inaccurate model absorption parameters, and possibly imprecise clearance parameters. Further optimization may improve fitting. Insight into the nature of gastrointestinal absorption could be gained from *in vitro* studies using Caco2 cells or other intestinal models. For elimination, hepatic intrinsic clearance is preferred over a rate constant. From the *in vitro* microsomal and S9 studies reported by Cao et al. (2008), data are provided that can be used to calculate metabolic intrinsic clearance. The Cao study demonstrated that the intrinsic metabolic clearance in a microsomal preparation was greater in humans than in rats and mice. However, concentration-dependent studies were not performed, so this publication does not provide support for the assumption of linear clearance.
- Clearance terms instead of first order rate constants (dependent on elimination and the apparent volume of distribution) would be more informative in the model. *In vitro* (Km/Vmax or intrinsic metabolic clearance) or derivation of intrinsic clearance from fitted clearance obtained from *in vivo* data may be used.
- The role of metabolites in toxicity is discussed in the draft assessment, but due to a lack of data not included in the model. This is appropriate, though limited information on metabolites in brain and other tissues (Pan et al. 2013) indicates they could contribute to the observed effects. The parent AUC dose metric would thus serve as an indicator of exposures to parent and metabolites, though not directly tracking the metabolites.
- Provision for tissue partitioning is mainly via *in silico* methods; more *in vivo* data would provide justification for these values should it become available in the future
- The mouse data are the least comprehensive, though EPA could re-evaluate whether the total radioactivity data in Guo et al. (1985) are consistent with the Sweeney et al. (2012) data.

Suggested Recommendations

- Revise the text to address the issues listed above as warranted, such as brain distribution and plasma protein binding.

3.2.2.Selection of Dose Metric

Charge Question 2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was

not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

For neurotoxicity, the choice of dose metric is clearly described (pages 2-8 and 2-9). However, the choice of dose metric for the prostatitis endpoint should be better described. The choice is reasonable and appropriate, given less than ideal data on the pharmacokinetic-pharmacodynamic (PK/PD) relationship for this endpoint. A PK/PD model likely would be driven by the concentration in brain that is responsible for the PD (neurotoxicity); brain RDX concentrations are derived from the blood-brain partitioning of RDX blood concentrations. Without brain RDX concentration data, plasma or blood is used as a surrogate for brain concentrations. The EPA's approach is adequately justified and appropriate, since limited PK data in mice, rats, and swine (Table C-1) and in a human (Woody et al. 1986) show concordance between blood and brain RDX levels over time following exposure, supporting the use of blood/plasma concentrations as a surrogate for brain concentrations, and for the use of plasma concentration-time curve AUC values as a dose metric.

AUC is representative of the average RDX plasma concentration over a dosing interval, i.e., 24-hour interval. Published 24-hour time courses of blood and brain RDX levels in rats (e.g., Bannon et al. 2009) appear to coincide with symptomatology, providing support for the use of AUC. It is appropriate to assume that seizures or hyperreactivity would be manifest as long as a threshold blood/brain concentration of RDX, e.g., 8 μg/g (Williams et al. 2011) has been reached or exceeded. Therefore, there is clear rationale for choosing AUC over peak plasma concentrations (C_{max}) values as the dose metric.

The POD_{HED} is presented in Table 2-2 of the draft assessment for both dose metrics for neurotoxicity, with the difference between C_{max} and AUC/24 hour values being relatively modest in the rat (~30%). It should be pointed out in the text on pages 2-8 that AUC appears to be a better representation of the adverse effect of interest than RDX concentration at a single point in time. Additionally, it should be noted that maximal plasma concentrations are not predicted well from the PBPK model, producing uncertainty in C_{max} values, and supporting the case for the use of AUC.

There does not appear to be an explanation for the choice of dose metric for the prostatitis endpoint, though some comments (e.g., AUC considered better estimated than C_{max} from PBPK model) in the discussion for neurotoxicity apply across endpoints. Again the differences in Table 2-2 are modest, and since this is an effect only observed in a chronic study, average daily AUC is an appropriate choice.

It is noted that although there are mechanistic data to support the role of RDX in neurotoxicity (convulsions) through binding to $GABA_AR$ (Williams et al. 2011; Williams and Bannon, 2009), the effect of RDX may be mediated by either parent compound or metabolites; as such, any PK parameter that measures parent compound plasma concentrations may not accurately predict toxicity.

The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a

dose metric for cancer endpoints. This decision is scientifically supported and clearly explained on pages C-30 and C-31. The mouse model is highly uncertain as discussed on pages 2-9 of the draft assessment.

Key Recommendation

- While current approaches for dose metrics are generally appropriate, the basis for the choice of dose metric for the prostatitis endpoint should be described.

3.2.3.Intrahuman Variation

Charge Question 2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

It is standard practice to adopt an intraspecies factor of 10 to account for potential differences in the toxicokinetics and toxicodynamics of a chemical in the absence of information about variability within human populations. There is a paucity of data on the toxicokinetics, toxicodynamics or toxicity of RDX in humans. Given these extreme data limitations and the likely toxicodynamic and toxicokinetic differences, it would not be appropriate to use a PBPK model and it is appropriate to use a full UF_H of 10.

Sensitivity analyses (described in Appendix C) showed that the PBPK model output was substantially impacted by bioavailability and by metabolic clearance. There are apparently no data to define the absorption phase following RDX ingestion by humans or animals. Toxicokinetic data for RDX elimination by humans are quite sparse. It appears from two studies (Bhushan et al. 2003; Major et al. 2007) that RDX metabolism in some mammals is mediated by cytochrome P450s (CYPs). As the activities of CYPs and other enzymes that metabolize xenobiotics vary significantly in the human population, the rate of metabolic clearance of RDX would also be expected to vary. Potential inter-subject differences in the formation of RDX metabolites may also contribute to uncertainty, should specific metabolites be associated with toxicities.

In light of the role of binding of RDX to the $GABA_AR$ in neurotoxicity, future data on inter-subject variability in receptor binding and response could identify and characterize sensitive subpopulations.

Key Recommendation

- None.

3.3. Hazard Identification and Dose-Response Assessment

3.3.1.Nervous System Effects

*3.3.1.1.*Nervous System Hazard

Charge Question 3a(i). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately

assessed? Is there an appropriate endpoint to address the spectrum of effects?

The SAB agrees that available human, animal, and mechanistic studies support the conclusion that nervous system toxicity is a human hazard of RDX exposure.

Human Studies
There is consistent evidence from more than 20 clinical case reports that exposure to RDX is associated with adverse neurological outcomes, particularly convulsions. Nevertheless, there are many and varied limitations to deducing the hazards of RDX solely based on such case reports. There is only one cross-sectional study (Ma and Li, 1993) that provides a snapshot of the potential neurotoxicity associated with inhalation exposure to RDX. In the translated publication, Ma and Li (1993) presented results at a single point in time from a neurobehavioral test battery (that assessed memory retention, simple reaction time, choice reaction time, letter cancellation, and block design (BD) which tested for visual perception and design replication, as well as ability to analyze spatial relationships) in two groups of workers (30/group) exposed to mean concentration of 0.407 or 0.672 mg/m^3 RDX in a Chinese plant. The average length of employment for these two groups were 11.8 and 9.8 years, respectively. The average length of employment for the control group of 32 people was 10.7 years. The results indicated that significant memory deficits and effects on visual perception and ability to analyze spatial relationships (BD) were associated with RDX exposure measured in air. However, this study has several significant limitations that impact any conclusions about RDX hazard solely based on its findings. The SAB's greatest concerns with this study are: 1) the omission of exposure levels in the "non-exposed" control group; 2) no attempt to control for confounders (non-occupational exposures, lifestyle, co-morbidity), and; 3) no rationale provided for subdividing the exposed cohort into two groups. Nevertheless, the outcomes on Composite Memory Retention Quotient and Composite Block Design score were greater than 15 points and greater than 2 seconds lower ($p<0.01$) than the control group, respectively. The statistical analyses performed seems appropriate, but 95% confidence intervals would have been helpful given that the magnitude of functional impairments across groups is within the High Average [110-119] and Average [90-109] range, measures typically associated with a 15% Standard Deviation. Other studies are generally supportive, with the strongest evidence for convulsions coming from investigations involving acute exposures (Testud, 1996; Hollander, 1969; Merrill, 1968).

Animal Studies
Several studies with rodents using oral gavage and dietary exposure over the acute (Burdette 1988), sub-chronic (Crouse et al. 2006; Von Oettingen, 1949) and chronic (Lish 1984, Levine 1983, Hart 1976) timeframes have consistently identified a broad range of neurological impairments, ranging in severity from irritability to tremors and other signs that may be considered prodromal of convulsions. Convulsive (seizure) activity is a common finding in most, but not all, studies. In addition to seizure activity, several of these studies (Levine et al.1990; Angerhofer et al. 1986; Levine et al.1983; Levine et al. 1981; von Oettingen et al. 1949) identified "less severe" neurological and behavioral impairments (e.g. hyperactivity and nervousness) that may be consistent with findings identified in the sparser literature on human exposures. Some of the animal study findings suggest that RDX appears to sensitize animals exposed at lower doses to subsequent seizurogenic stimuli, including electrogenic, audiogenic, and chemical kindling.

Mechanistic Studies

The neurotoxicity profile of RDX is consistent with that of a centrally acting excitotoxicant. There is ample evidence of a direct interaction of RDX with $GABA_AR$ in the mammalian central nervous system. RDX blocks GABA-activated chloride ion currents and the inhibitory postsynaptic potentials (IPSPs) that form critical inhibitory networks throughout the brain. The available data do not preclude the influence of other unstudied receptors for RDX, but also implicate the limbic system, including the amygdala, as particularly sensitive targets of RDX. The potency of RDX as a $GABA_AR$ blocker is relatively low when compared to other convulsant agents. For instance, picrotoxin (PTX) has a 100-fold lower inhibition constant or K_i (i.e. 100x more potent) than RDX at binding to $GABA_AR$, with K_i's of 0.2 vs. 21 μM, respectively (Williams et al. 2011). The lower potency of RDX extends to the concentrations needed to inhibit chloride ion currents in whole cell voltage clamp experiments and inhibitory postsynaptic current (IPSC) events, which typically require greater than 10 μM. Also relevant to the RDX mechanism of action and its potential importance to long-term behavioral toxicity is the observation that the inhibitory actions of RDX on seizure-like neuronal discharges can be measured in the basolateral nucleus of the amygdala (Williams et al. 2011). In contrast, evidence supporting a direct role for glutamate in the effects of RDX is limited, and a basis for excessive glutamate stimulation in the draft assessment is weak, if not unfounded. However, the overall excitation within neuronal networks of the adult mammalian brain is controlled primarily, though not exclusively, by the balance of glutamatergic (excitatory) and GABAergic neurotransmission among interconnected circuits, and thus are inextricably linked.

Conclusions

Regarding nervous system hazard identification, the available human, animal, and mechanistic studies support EPA's conclusions that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. Furthermore, RDX-induced convulsions arise primarily through a rapid mode of action resulting from RDX-induced $GABA_AR$ blockade. Despite the limitations of the Ma and Li (1993) study, the sum of the evidence from clinical case reports, experimental animals, and mechanistic studies of RDX indicates there is sufficient evidence to support the EPA conclusion. Therefore, RDX should be considered a *potential convulsant to humans* who are at risk for exposures to RDX.

The evidence presented in the draft assessment, however, does not fully depict RDX's hazards to the nervous system. The SAB notes that convulsions in rodents only provide a limited spectrum of potential human hazard, with convulsive or non-convulsive seizures, epileptiform discharges (Fernandez et al. 2015; Wyllie and Devinsky, 2015), reduction in seizure threshold, subchronic sensitization, and neuronal damage all being part of the spectrum of RDX's nervous system hazards. Further evaluation or explanation should be provided in the report for these additional potential endpoints. With respect to whether all hazards to the nervous system were adequately assessed, the measure of abnormal electrographic activity or seizure-like activity in specific brain regions may be a more sensitive indicator of neurotoxicity than the potential of RDX to elicit subtler neurological impairments such as cognitive deficits and/or behavioral abnormalities.

Although endpoints such as convulsions, tremors and aggression are appropriate as part of the spectrum of effects, it is important to note that the functional observation battery (FOB) data pre-

sented in Crouse et al. (2006) are not sufficiently sensitive to detect neurobehavioral consequences produced by chronic/subchronic doses of RDX over prolonged periods, especially during pregnancy. Moreover, tests designed to detect subtle developmental neurotoxicity during the perinatal-weaning period have not been reported to date. Most of the FOB methods used were observational and highly descriptive, and considered blunt instruments likely to have missed relevant neurological impairments, if present. The data presented by Crouse et al. (2006) sets a range and NOAEL for convulsion, but no conclusions can be reached about the lower limit of subconvulsive doses that are without frank neurological impacts, including fine psychomotor impairments, anxiety and social impairments, decreased executive functioning and long term memory. These concerns are compelling because of more recent peer-reviewed published data indicating that subconvulsive doses of either bicuculline (which has a similar mechanism of action to RDX) or domoic acid (which has agonist activity on glutamate transmission) cause developmental and behavioral impairments at doses below those that cause convulsions (Salari and Amani, 2017; Gill et al. 2010). GABAergic and glutamatergic neurotransmission are inextricably linked, not only in regulating excitability of the adult brain, but the fact that their balance throughout perinatal development provides essential developmental cues that refine functional neural connectivity. The lack of scientific information about the influences of RDX on this balance is a major uncertainty. Thus, the SAB concludes that there remains significant uncertainty about the developmental neurotoxicity of RDX. Additional studies addressing cognitive and behavioral effects of RDX would assist in assessing endpoints less severe than convulsions. Although there are data from existing animal studies showing changes in behavior, the data are not sufficiently robust to evaluate dose-response relationships, and animal data on cognitive changes are lacking. Given these limitations, additional studies measuring other neurological endpoints are needed to address the complete spectrum of effects.

Key Recommendations

- Lack of studies on neurodevelopmental toxicity, as well as cognitive and behavioral effects of RDX should be recognized in the assessment (see discussion in Section 3.3.1.4, Database Uncertainty Factor (UF_D).

3.3.1.2. Nervous System-Specific Toxicity Values

Charge Question 3a(ii). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

The selection of studies reporting nervous system effects is scientifically supported, clearly described, and provides sufficient information to identify the central nervous system as a primary toxicological target for exposures to RDX. Based on a review of the scope of the search strategy and the process for identifying studies that report health effects and meet appropriate standards of quality for conduct, design, and reporting, the SAB concludes that the most reliable scientific information has been accessed for this draft assessment.

For assessment of nervous system effects, the convulsion endpoint is appropriate for revealing the hazards of RDX delivered by oral gavage administration (Crouse et al. 2006; Cholakis et al. 1980) or the dietary route (Levine et al. 1983; Lish et al. 1984).

However, the available data from animal studies with RDX do not adequately address the potential effect of low-level exposure(s) of RDX either through life stages, and importantly, during the highly susceptible perinatal period (see Section 3.3.1.1). Based on the current state of the science with other compounds known to elicit seizures by similar or functionally related mechanisms, the SAB cannot discount whether RDX is capable of producing subtle, yet relevant, behavioral, psychomotor, or cognitive outcomes. The SAB generally agrees that the balance/imbalance of neuronal excitation/inhibition during the perinatal and post-weaning periods of development have profound and measurable influences on the functional and anatomical integrity of developing neuronal networks. This not only impacts behavioral, psychomotor, and cognitive outcomes throughout the lifespan, but also promotes significantly greater susceptibility to subsequent exposures to other seizurogenic chemicals or physical triggers of convulsion (Stamou et al. 2013; Lee et al. 2016; Meunier et al. 2017). There is a wealth of peer-reviewed experimental evidence showing that even modest impairments in the excitation/inhibition balance of developing neuronal circuits, whether originating from genetic mutations, chemical exposures, or their combination can effect long-lived (possibly permanent) changes in behavioral, psychomotor, and cognitive outcomes. This is particularly important for chemicals that interfere with GABAergic neurotransmission. It is also important to emphasize the developmental transition of $GABA_A$ receptor from excitatory to inhibitory neurotransmission during the perinatal/postnatal period, a shift that affords additional complexity to how RDX exposures alter neurological outcomes. This is especially important since RDX has been shown to interact in a competitive manner with picrotoxin at $GABA_A$ receptors of basolateral amygdala (BLA), but once it alters their function, its actions are not reversible (Williams et al. 2011). Therefore, there are major gaps in our knowledge about exposures to subconvulsive doses of RDX and their possible neurological ramifications, especially during the perinatal and early weaning periods of development. Clearly, such exposures are possible and relevant, and could have consequences not only to individuals directly exposed to RDX, but also those exposed transplacentally and/or during lactation. Additional developmental neurotoxicity studies need to be conducted in animals to address these gaps, including test batteries to detect potential fine psychomotor impairments, anxiety and social impairments, decreased executive functioning and long-term memory.

Biological plausibility for such detrimental actions comes from a rich literature demonstrating that developmental exposure *in vivo* and *in vitro* to seizurogenic chemicals have potent influences on outcomes relevant to developmental neurotoxicity at concentrations below those that elicit convulsions. These agents have been shown to influence behavioral, psychomotor, and cognitive outcomes. Examples relevant to RDX include the $GABA_AR$ antagonist bicuculline (Grasso et al. 2016; Nasehi et al. 2017; Salari et al. 2017), and domoic acid (Costa et al. 2010; Doucette et al. 2016; Grant et al. 2010; Hiolski et al. 2016; Marriott et al. 2016; Mills et al. 2016; Zuloaga et al. 2016). Lastly, Zhang and Pan (2009) provided strong evidence that adult mice fed diets with RDX at a subconvulsive dose of 5 mg/kg for 28 days resulted in significant changes in key miRNA brain transcripts that are related to neurological and metabolic functions, and also were changed in a tissue-specific manner. Such effects are likely to exert long-lived consequences on brain development should they occur during the perinatal period (Hu and Li, 2017).

The differences in toxicokinetics of RDX exposure by gavage versus dietary administration are clear, and must be accounted for when predicting risk. Animal studies reporting effects on neurological health utilized gavage or dietary route of administration. The evidence indicates that the gavage route results in higher peak blood and brain levels of RDX than the dietary route, and that the rate of rise in blood and brain levels is faster with gavage. Gavage results in more reliable and consistent dose to blood and brain than dietary intake. Moreover, incidences of convulsions were not reported in most dietary studies (Levine et al. 1983; von Oettingen et al. 1949). The SAB concludes that although dietary intake is the most likely route of exposure for the general population, it is appropriate to consider the dose–response data reported in the Crouse et al. (2006) study as a relevant model. In fact, the Crouse study produced the best RDX dose-response data available for convulsion. The SAB recognizes that the use of a gavage study rather than a dietary study allows for some unquantified margin of safety in the RfD.

EPA also used the incidence data for convulsions in pregnant dams dosed by gavage with RDX from gestation days 6 – 19 from the teratology study of Cholakis et al. (1980) for dose-response assessment. The candidate POD and RfD derived from the Cholakis et al. study was 5 times lower than those derived using data from the subchronic study of Crouse et al. (2006). This may indicate that pregnancy is a sensitive window for neurotoxicity in the adult. Or, it may indicate that the higher sample size of 24 to 25 per dose used by Cholakis et al. (1980) was sufficient to detect convulsions at a lower dose than the Crouse study, which had a sample size of 10 animals per dose. However, considering the uncertainty regarding the actual doses administered in the Cholakis et al. study and other study limitations noted by EPA concerning quantification of the dose-response relationship, EPA elected to use the Crouse study as the basis of the proposed RfD value. The SAB supports this decision, as detailed below and in Section 3.4.1.

The noted limitations in the Cholakis study compared to the Crouse study included in the report are the lower purity test compound, the shorter, 14- day dosing regimen compared to 90 days, and use of three widely spaced (order of magnitude) dose groupings versus five tightly spaced dose groupings in the Crouse study. In principle, all of these differences can impact the accuracy of a POD calculation. It is worth noting, however, that after subtracting out water, the purity in Cholakis was 90% compared to 99.9 % in the Crouse study, and the impact of this difference on study findings cannot be ascertained. A significant limitation of the Cholakis et al. (1980) study that was not described in the draft assessment was the difficulty encountered keeping the chemical uniformly suspended in solution. In both the Cholakis et al. (1980) teratology study and the Crouse study, the doses of RDX were administered in a methyl cellulose / Tween 80 vehicle as a suspension. The assay results for the dosing suspensions presented in the appendix of the Cholakis report demonstrate high variability and the study authors acknowledged “maintaining uniform suspensions was not always easy.” When the same nominal concentration was assayed repeatedly, it showed wide variation in RDX content (33% to 500% relative to nominal), although the RDX concentration of one of the assayed dosing suspensions was much higher (500%) than nominal and skewed the range of variability. Most assayed dosing suspensions were lower than nominal. Nonetheless, the difficulty in maintaining uniform suspension introduced considerable uncertainty in the actual doses administered in the Cholakis study. Less variability in RDX dose suspensions was observed in the Crouse study because each dose suspension was mixed using a magnetic stirring bar until a uniform suspension was obtained, and continued to be mixed

each day during the dosing procedure. Since these measures were taken by Crouse et al. to reduce the variation in dosing suspensions, it is likely that the intended dose levels were more accurately administered in the Crouse study compared with the Cholakis study. The problem maintaining uniform dose suspensions should be identified in the EPA assessment as a critical study limitation that increases uncertainty in deriving the RfD based on the Cholakis et al. (1980) study. However, the SAB also notes that Cholakis et al. (1980) observed convulsions in a pregnant dam at a dose (2 mg/kg-day) lower than the LOAEL of the Crouse study (8 mg/kg-day) in male and nonpregnant female rats. Further, in the Angerhofer et al. (1986) teratology study, one death was reported in the dams at 2 mg/kg-day and one death at 6 mg/kg-day, although the authors did not report whether convulsive symptoms occurred prior to death. Although the evidence is soft, these findings raise the possibility that pregnancy may be a sensitive physiological state for the neurotoxicity of RDX. Overall, considering all of the above factors, the SAB concludes that it is appropriate to give more weight to the Crouse study with respect to the quantitative dose-response analysis.

The SAB agrees that the characterization of convulsions as a severe endpoint, and its potential relationship to mortality, are appropriately described. Based on the available data, death may occur without seizure or convulsions, although this may simply be due to a low frequency of observations. However, based on the current state of science (including the epilepsy literature), death is not a necessary outcome of seizures or convulsions, and is driven by abnormal electrographic patterns in the brain. While the relationship between convulsions and mortality is unclear in the overall scheme of assessment of neurotoxicity endpoints for RDX, it is nonetheless appropriate to conclude that convulsions, as characterized in the draft assessment, represent a reasonable severe endpoint for human health risk assessment. In addition, more consideration should be given to available data on fatal outcomes and the possibility that mortality may arise from non-nervous system factors or hazards.

Key Recommendation

- The problem maintaining uniform dose suspensions should be identified in the EPA assessment as a critical study limitation that increases uncertainty in deriving the RfD based on the Cholakis et al. (1980) study.

Suggested Recommendation

- More consideration should be given to discussing available data on fatal outcomes and the possibility that mortality may arise from non-nervous system factors or hazards.

3.3.1.3. Points of Departure for Nervous System Endpoints.

Charge Question 3a(iii). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

<u>Convulsion Endpoint:</u>

The SAB finds that the selection of convulsions as the endpoint to represent nervous system hazard for RDX is scientifically supported and clearly described. The evidence indicates that convulsions are the most biologically significant endpoint that has been reasonably and reliably measured. However, the SAB notes that evidence from other seizurogenic compounds with similar modes of action suggests that there are other, generally subclinical cognitive and behavioral neurological effects that occur at lower doses. It is likely that such effects also occur for RDX, although data to firmly establish this point are not currently available. For compounds such as bicuculline, triggering of abnormal biochemical, electrographic patterns and/or abnormal connectivity measured by magnetic resonance imaging (MRI) or positron emission tomography (PET) approaches can be demonstrated to occur at doses below those that cause seizures (Bruyns-Haylett et al. 2017; Galineau et al. 2017; Nasrallah et al. 2017; Takahashi et al. 2017). Moreover, although it is difficult to extrapolate across chemicals with the same mode of action in terms of potency to induce a specific effect, the SAB provides the following comparison to exemplify this point. For bicuculline, a $GABA_A$ receptor antagonist like RDX, White et al. (2008) reported that the subcutaneous dose provoking seizures in 97% of adult mice is 2.70 mg/kg, whereas Salari and Amani (2017) showed developmental and behavioral impairments at subconvulsive doses of 300 µg/kg, but not 150 µg/kg, via subcutaneous administration to neonatal mice. Thus, in this example, the difference between a developmentally neurotoxic dose and a convulsive dose is ~10-fold. Although one cannot directly extrapolate this dose comparison to RDX, it provides some indication of the possible difference between a developmentally neurotoxic dose and a convulsive dose for a chemical that acts in the same manner as RDX.

As such, the SAB agrees that the likely dose range between convulsion and other nervous system effects can be addressed using the UF adjustments.

POD Calculations:

The draft assessment determined that the incidence data for convulsions from Crouse et al. (2006) and Cholakis et al. (1980) were amenable to BMD modeling. PODs based on a BMR of 1% extra risk for convulsions were calculated for both studies. In addition, a POD based on the NOAEL for convulsion from the two-year dietary study of Levine et al. (1983) was also derived. The SAB questions whether the Cholakis et al. data is appropriate for BMD modeling or identification of a POD, given the concerns identified in Section 3.3.1.2 and in response to charge question 4a presented in Section 3.4.1. The SAB concludes that the other PODs for convulsions were clearly described and correctly calculated. However, the SAB questions the use of BMR of 1% extra risk for convulsions, as discussed in this section.

HED Calculations:

The SAB agrees that the calculation of the HEDs for these studies is scientifically supported and clearly described in the assessment. EPA estimates the HED by assuming the equivalent pharmacokinetic potency of equivalent rat and human arterial blood concentrations of RDX. The concentration of RDX as a function of time following dosing is generated using a PBPK model, and the effective concentration is estimated as the AUC of concentration and time. The SAB endorses this approach. The SAB agrees that, given the binding of the parent compound to the $GABA_AR$, a dose metric for the parent compound is appropriate, though it also may be serving as a surrogate if any metabolites also have that activity. The AUC is a more appropriate choice

than C_{max} to estimate the effective concentration due to the uncertainties in the parameterization of the model for absorption.

Benchmark Response Level for Convulsions:

The SAB identifies the following concerns regarding EPA's use of a BMR of 1% in the benchmark dose model of the dose-response data from Crouse et al. (2006). Based on EPA's *Benchmark Dose Technical Guidance* (U.S.EPA, 2012a), the "standard reporting level" (although not *per se* the default) BMR for quantal data (such as those for the incidence of convulsions) is 10%. In that guidance, EPA suggests conditions that would justify BMR values less than 10%. The justification given in the guidance for applying a smaller BMR is "biological considerations" of the endpoint being modeled. EPA's guidance does, in fact, identify "frank effects" as an example of such biological considerations for choosing a BMR of "5% or lower." In addition, a 1% BMR is recommended for epidemiological data. However, the guidance also points out that "…if one models below the observable range, one needs to be mindful that the degree of uncertainty in the estimates increases. In such cases, the BMD and BMDL can be compared for excessive divergence. In addition, model uncertainty increases below the range of data." In its clarification of this choice to the SAB, the EPA stated that the BMR of 1% was chosen based on biological considerations as given in its Benchmark Dose Technical Guidance. Specifically, EPA stated that this BMR was chosen to address the fact that the endpoint being modeled, in this case convulsions, is a frank effect. The SAB acknowledges that the convulsions observed by Crouse et al. (as well as by Cholakis et al.) indeed, repesent a frank effect, and the SAB is sensitive to the need to provide an adequate margin of safety to protect against even a low frequency of occurrence of this effect. However, the SAB finds that EPA's Benchmark Dose Technical Guidance is vague on how "biological considerations" should influence the benchmark dose modeling. The lack of clarity in the use of this term, and the absence of guidance as to how "biological considerations" should be applied in choosing a BMR, makes its application subjective.

Benchmark dose modeling was developed to address the constraints placed upon dose-response assessment by the use of only study-specific, and dose-specific NOAELs and LOAELs, and to fully utilize the data on response to dosing. The purpose of benchmark dose modeling is to derive PODs from study data that are more generalizable to the inherent dose-response of a given chemical than are the study's NOAEL or LOAEL. Benchmark dose modeling is viewed primarily as a process for modeling the dose-response *per se* using few, if any, assumptions that are extraneous to the data (except in rare cases where mechanistic information may inform the shape of the dose-response curve). Consistent with this view, the BMR should be strongly linked to the nature of the dose-response data. Hence, the caution expressed in EPA's benchmark dose guidance that, "…if one models below the observable range, one needs to be mindful that the degree of uncertainty in the estimates increases. In addition, model uncertainty increases below the range of data." Thus, the BMR should be close to (although not necessarily within) the observable data. The BMR determines the "distance" between the observable data and the BMD. As indicated in the EPA guidance document, the greater the "distance" between the observable data and the BMD, the greater the statistical uncertainty in the fit of the model at the BMD and, therefore, the greater the difference between the BMD and the BMDL. The computations presented in Table 1 show that the response at the LOAEL for the Crouse et al. (2006) study is 15% and for the Cholakis et al. study is 4%. Thus, a BMR of 1% corresponds to a response that is a factor of 15 below the lowest observed responses for the study chosen for estimation of the POD.

Table 1. LOAELs and Percent Response at LOAELs for Crouse et al. (2006) and Cholakis et al. (1980)

Study	n/dose group	LOAEL	Percent response at LOAEL
Crouse et al. (2006)	10 rats/sex/dose – group	8 mg/kg/d	15%
Cholakis et al. (1980)	24-25 pregnant rats/dose group	2 mg/kg/d	4%

Table 2 below presents the BMDs for the Crouse et al. (2006) study that would result from BMRs of 1%, 5% and 10%. The EPA benchmark dose guidance suggests looking at the BMD/BMDL ratios, also provided in Table 2, resulting from each BMR but provide no guidance on what constitutes a ratio indicative of unacceptable statistical uncertainty, and thus a BMR too low to be supported by the data. Note that the BMDL at a BMR of 1%, the EPA estimated POD, is roughly 4 times smaller than the BMDL at a BMR of 5%.

Table 2. Comparison of BMDs and BMDLs at different BMRs for Crouse et al. (2006)

Study	**BMR**	**BMD** (mg/kg-d)	**BMDL** (mg/kg-d)	**BMD/BMDL**
Crouse et al. (2006) LOAEL = 8 mg/kg-d	1%	3.02	0.569	5.3
	5%	5.19	2.66	2.0
	10%	6.60	4.59	1.4

The EPA's justification for the use of a BMR of 1% versus 5% or 10% is based on its interpretation of their Benchmark Dose Technical Guidance (USEPA, 2012a), which states that "for standardization, rounded values of 1%, 5% and 10% have been used" and that a BMR of "5% or lower" may be warranted for frank effects. The guidance does not, however, specify 1%. However, EPA does not focus on the other aspect of the choice of a BMR that is highlighted in the guidance, that of closely adherence to the data. The contention raised by the SAB is less that EPA needs to provide a justification per se for their choice of a BMR of 1% - as the guidance does provide opportunities where that can be used, but rather, that the guidance itself does not provide a basis for balancing the competing concerns of frank effect, and adherence to the data. In this respect, EPA's justification for choosing a BMR of 1% should specifically address how

the tradeoff between the nature of the effect and the nature of the data were addressed in the assesment and how it should be addressed in more general terms. In addition, and in a closely related consideration, EPA should also address how and when the nature of the frank effect is most appropriately addressed in the UFs as opposed to the benchmark dose modeling.

BMDL for Convulsions:
The calculation of the lower bound on the benchmark dose (BMDL) for convulsions is appropriate and consistent with EPA's Benchmark Dose Guidance. For the parameters specified by EPA (including the choice of a BMR of 1%), the benchmark dose is calculated per EPA's benchmark dose guidance. The choice of the model from among the available dose-response models is appropriate.

Key Recommendations

- EPA should consider using a BMR of 5% for their dose-response modeling of the Crouse et al. (2006) data, while addressing the uncertainty of using data on a frank effect (convulsions in this case) as the basis of an RfD with a larger database uncertainty factor.
- If EPA decides to use a BMR of 1% for the dose-response assessment using Crouse et al. (2006), EPA should justify why the greater conservatism in risk assessment required for a frank effect (due to the lack of incidence data for less severe endpoints) is better dealt with through a lower BMR rather than through application of UF_D.
- If EPA decides to use a BMR of 1% for the Crouse et al. (2006), EPA should provide clear justification for why a 1% BMR is more appropriate than a 5% BMR for RDX, given the greater uncertainty introduced into the dose-response assessment for RDX using a BMR of 1%.

3.3.1.4.Uncertainty Factors for Nervous System Endpoints

Charge Question 3a(iv). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3?

EPA applied Benchmark Dose Software models to data from two gavage studies in rats (Crouse et al, 2006; and Cholakis et al. 1980) to derive a benchmark dose for a 1% response rate ($BMDL_{01}$) as a point of departure for effects on the nervous system, following Human Equivalent Dose (HED) adjustment. A third data set (Levine et al. 1983) in rats was evaluated using the NOAEL approach. The toxicological endpoint in all cases was convulsions. EPA applied UFs to the HEDs to derive the proposed RfD for nervous system effects.

Interspecies Uncertainty Factor (UF_A)
An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the points of departure (PODs), in this case the human equivalent dose for a 1% response rate, to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolating from average animal models to average humans not accounted for by the toxicokinetic modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive a human equivalent dose, and available data are not sufficient to define quantitative toxicodynamic

differences between species. The SAB agrees that the UF_A of 3 is appropriate and clearly described.

Subchronic to Chronic Uncertainty Factor (UF_S)
EPA chose the subchronic study of Crouse et al. (2006) to derive a RfD for nervous system effects and a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure, primarily because as stated on page 2-11, lines 22-24 of the draft assessment, "in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in the chronic studies".

The SAB has concerns about the use of a UF_S of 1. An *in vitro* assay of GABA activity showed that the effects of RDX were not reversible following compound wash out (Williams et al. 2011). Furthermore, in a 14-day range finding study in Sprague Dawley rats, Crouse et al. (2006) observed convulsions (incidence and severity not reported) at doses of 17 mg/kg-day and above, and no convulsions at 8.5 mg/kg-day (male: 0/6; female 0/6). In a 90-day study on F344 rats, the same investigators using the same dosing method reported that convulsions were elicited at 8 mg/kg-day (male: 1/10; female: 2/10) and above. Though the apparent greater sensitivity to convulsions in the longer exposure study may be due to a larger number of animals per group (10 animals/group vs. 6 animals/group in the 14-day study), or rat strain differences, the finding of convulsions at a lower dose in the 90-day study by the same investigators using the same procedures for administering RDX may reflect the influence of the longer exposure period. These observations raise the possibility of progressive, possibly cumulative effects on GABAergic neurotransmission that are not predicted by either RDX pharmacokinetics in the blood nor by the total levels of RDX in the brain, as only a minor fraction of total RDX in the brain would be bound to the $GABA_AR$ pool. If progressive effects do occur, there may be some compensation in the balance of excitatory and inhibitory neurotransmission, but the potential of such compensation to mitigate effects of RDX and the impact of compensation on the organism are unclear.

The SAB also has concerns about part of the EPA's rationale for using a UF_S of 1, namely that "in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in the chronic studies" (Section 2.1.1, p 2-11 in the draft assessment). The three studies used to generate PODs were a gestational study with 14-day gavage exposure to pregnant dams (Cholakis et al. 1980), a 2-year dietary study in male and female rats (Levine et al. 1983), and a 13- week gavage study in male and female rats (Crouse et al. 2006). As discussed in Section 3.3.1.2, pregnant dams in the Cholakis et al. gestational study may be a potentially sensitive subpopulation that is not readily comparable to non-pregnant animals. Thus, the 14-day Cholakis study and 90-day Crouse study or longer term dietary studies should not be compared to evaluate the effect of exposure duration on convulsant dose.

As EPA notes in the discussion of studies and in Appendix C of the Supplemental Information document, differences in the method of dose administration, the physical form of RDX, including particle size, and/or dose matrix in the dietary studies and gavage preparations may influence the rate of absorption and internal dose, and may partly explain the differences in neurotoxic

symptoms reported in the studies of varying duration, both dietary and gavage. RDX administered orally as a coarse particle preparation was shown to be more slowly absorbed than as a fine particle preparation (Schneider et al. 1977), thus influencing the kinetics of RDX. Differences in particle size in the 13-week dietary study in mice of Cholakis et al. (RDX particle size of about 200 µm), and the 2-year dietary study in the same strain of mice by Lish et al. (RDX particle size less than 66 µm), may partly explain why no convulsions were reported at higher doses in the 13-week study, but observed at lower doses in the 2-year study. Overall, differences in study population, dosing preparations, route of administration, and other methodological considerations make comparisons across studies for the purpose of evaluating effect of exposure duration difficult, if not impossible. Thus, EPA's statement that effects were seen at lower doses in shorter duration exposures than in the chronic studies is inappropriate. In making comparisons of the toxicity of RDX after different durations of exposure, factors such as, particle size, dosing method, and dose matrix, that are known to influence rate of gastrointestinal absorption and/or bioavailability, should be addressed in the discussion where possible. Note that the test material used in the key study of Crouse et al. (2006), although of higher purity than most other studies, was not characterized with respect to particle size.

The SAB recognizes that the NOAEL for convulsions in the 2-year dietary study in rats (Levine et al. 1983) was 8 mg/kg-day, which was 2-fold higher than the NOAEL of 4 mg/kg-day for convulsions in the 13-week gavage study in rats (Crouse et al. 2006); and may be the primary reason EPA used as a basis for the application of a UF_S less than the default value of 10. However, the differences in the observed convulsant doses may be due to differences between dietary and gavage administration. As discussed in Section 3.3.1.2, RDX administered via gavage results in a more reliable and consistent dose to blood and brain than dietary intake. Moreover, the 2-year dietary study was not designed for dose-response assessment for convulsions in exposed animals, and incidences of convulsions were not reported. Thus, occurrences of convulsions might have been missed during the course of the study. This makes it even less appropriate to compare with the Crouse study, which was designed to evaluate incidence of convulsions.

The case for the value of the UF_S is less clear to the SAB than that for the UF_D discussed in the following section. Thus, the SAB recommends that EPA reconsider the UF for subchronic to chronic extrapolation, and at a minimum, provide stronger justification for a UF_S of 1. A UF_S of 1 means that there is no uncertainty in extrapolating the POD from a 90 day study to a POD for chronic exposure. As noted above there is some evidence that slow reversibility of binding of RDX to the $GABA_AR$ may provide for cumulative effects on inhibitory neurotransmission. Further, the uncertainty in dose rates received by animals in the various studies due to particle size and related issues makes cross-study comparison of the effects of duration of exposure inappropriate.

LOAEL to NOAEL Uncertainty Factor (UF_L)

The UF_L is meant to account for uncertainties in extrapolating from a LOAEL to a NOAEL when estimating an RfD. EPA applied a UF_L of 1 because the BMDL was used as a point of departure in Crouse et al. (2006) and in Cholakis et al. (1980), and a NOAEL was used as the point of departure in Levine et al. (1983). Thus, no extrapolation from a LOAEL to a NOAEL was needed. This is standard risk assessment practice and the Panel agrees that this choice is appropriate and clearly described.

Database Uncertainty Factor (UF_D)

The EPA applied a UF_D of 3 in developing an RfD based on neurotoxicity to help account for database deficiencies. The SAB has several concerns regarding the large amount of database uncertainty for RDX including lack of developmental neurotoxicity testing, frank effect as a basis for the RfD with no available incidence data for less severe neurotoxicity, and proximity of the dose inducing convulsions with that inducing mortality. The SAB recommends increasing the UF_D from 3 to the default value of 10, per EPA risk assessment guidelines (U.S. EPA, 2002).

The SAB is concerned that there is limited information available to understand developmental neurotoxicity of RDX. Transplacental and lactational transfer of RDX in rodents has been observed (Hess-Ruth et al. 2007), and therefore, there is potential exposure to the developing fetus and infant from maternal exposure. It is worth noting that Hess-Ruth et al. (2007) concluded that developmental neurotoxicity studies should be conducted for RDX, but apparently, this has not been done. EPA noted that the two-generation reproductive and developmental toxicity study of Cholakis et al. (1980) did not report effects in the offspring at doses lower than maternally toxic doses. However, the study only looked at histopathology of 32 organs/tissues of the F2 pups at weaning. Histopathology of the F1 offspring were not examined. This study did not assess developmental neurotoxicity in the offspring. The draft assessment indicates that the existing literature did not demonstrate early life stage as a sensitive subpopulation, but this was not fully evaluated in animal studies and cannot be evaluated with the available human data. There was one case report involving one child poisoned by RDX, but this one case study does not provide evidence regarding the influence of age at exposure on toxicity.

RDX interferes with neurotransmission by binding at the $GABA_AR$, and acting as an antagonist inhibiting GABAergic neurotransmission. GABA is a major inhibitory neurotransmitter in the adult brain. However, GABAergic systems play another role in vertebrate brain development acting as an excitatory neurotrophic factor contributing to processes involved in neurodevelopment (see Rivera et al. 1999; Kim et al. 2012). There is evidence that exposure of early postnatal rodent hippocampal slices to a GABA antagonist (bicuculline) reduces GABAergic neuroactivity, affects the regulation of GABAergic inhibitory synapses and increases their density in the hippocampus (Marty et al. 2000). The hippocampus is involved in seizure development in humans with epilepsy, so these results seem pertinent. There is evidence that drugs that act through the $GABA_AR$ as GABA agonists can also cause neurodevelopmental disorders (see review by Creeley, 2016). These lines of evidence point to potential window(s) of susceptibility in the developing brain to chemicals interfering with GABAergic systems. Additional discussion is provided in Section 3.3.1.2.

Additional evidence prompting concern for developmental neurotoxicity is found in the section of the draft assessment on the mode of action of RDX neurotoxicity. The draft assessment cites a study (Zhang and Pan, 2009) reporting that RDX upregulates 3 microRNAs that affect brain-derived neurotrophic factor (BDNF) in the brains of mice fed 5 mg RDX/kg diet (estimated doses 0.75 to 1.5 mg/kg-day; Bannon et al, 2009). As EPA notes, BDNF is a member of the neurotrophin family of growth factors, and promotes the survival and differentiation of existing and new neurons. As such, disruption of BDNF regulation may result in developmental deficiencies in the brain. This provides additional indirect evidence raising concern for potential developmental

neurotoxicity of RDX. Zhang and Pan (2009) provided strong evidence that adult mice fed RDX at a subconvulsive dose of 5 mg/kg for 28 days resulted in significant tissue-specific changes in key miRNA brain transcripts related to neurological and metabolic functions. Such effects could have long-lived consequences on brain development should if present during the perinatal period (Hu 2017). EPA does not discuss the role of GABAergic systems in neurodevelopment and the potential for interference with this system by RDX (or other compounds with similar molecular mechanisms) to induce developmental neurotoxicity, an omission that should be rectified (see recommendation under Section 3.1). Until there are adequate developmental neurotoxicity studies on this compound, the potential for developmental neurotoxicity as an outcome of RDX exposure remains a significant data gap.

As discussed in Section 3.3.1.3 for bicuculline, a chemical with the same mode of action as RDX, the subcutaneous dose that causes neurodevelopmental effect is about 10 fold lower than the convulsion dose (although one cannot extrapolate directly across chemicals with the same mode of action).

The SAB notes that EPA chose to model a $BMDL_{01}$ rather than a $BMDL_{05}$ because of the convulsion endpoint. The SAB, as discussed in response to Charge Question 3a(iii) (Section 3.3.1.3), has concerns about the use of a BMR of 1% because of the degree of uncertainty introduced in the benchmark dose analysis, and suggests that EPA consider the use of a BMR of 5%. While recognizing the need for conservatism in the development of the RfD given the use of a frank effect (convulsions) as the critical effect, the SAB suggests that rather than trying to capture this conservatism in the benchmark dose analysis of the Crouse study data, the EPA consider the UF_D a more appropriate framework to provide protection. Further, choosing a lower BMR from a study in adult animals does not account for the potential of widely different toxicodynamics as a function of age at the time of exposure. There are other considerable uncertainties in the database including the lack of testing for developmental neurotoxicity and proximity of convulsive doses to lethal doses. Therefore, the SAB concludes that the full default UF_D of 10 should be used with a BMR of 1% or 5%, and the use of this uncertainty factor should be sufficient to account for the uncertainty caused by the use of a 5% BMR for a frank effect. As noted already, use of a gavage study rather than a dietary study as the basis of the RfD provides some unquantified margin of safety due to the higher blood levels achieved after bolus dosing.

EPA's BMD modeling (see Appendix D in Supplemental Information for the draft RDX assessment) of the mortality data also indicates that convulsive doses and lethal doses are approximately the same. The $BMDL_{01}$s for lethality from studies amenable to modeling overlay the $BMDL_{01}$s for convulsions. Note that the Crouse et al. (2006) study authors state that their study provides a NOAEL of 4 mg/kg-d for lethality. This is the same NOAEL for convulsions. Thus, mortality occurs in the same dose range as convulsions. EPA does not use lethality as an endpoint for a chronic RfD, yet in the case of RDX, lethality and convulsions occur at the same doses. This finding provides additional compelling support for using a UF_D of 10 rather than 3. Given the potential for neurodevelopmental toxicity of RDX through interference with GABAergic systems and other pathways, the proximity of lethal doses to convulsive doses, and the lack of incidence data on less severe neurotoxic effects of RDX, the SAB strongly recommends that EPA use a UF_D of 10 rather than 3.

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population. Although a PBPK model was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose, EPA noted that not enough toxicokinetic data were available from human studies to quantify differences among humans.

The SAB agrees that the UF_H needs to account for both toxicodynamic and toxicokinetic variability among humans, and that not enough data were available to quantify toxicokinetic or toxicodynamic differences among humans. EPA used the standard default UF_H of 10, which is typically viewed as a composite of a half-log for toxicokinetic differences and a half-log for toxicodynamic differences. Toxicokinetic differences among humans can be related to age, pregnancy, illness, medication use, other chemical exposures, and so on. In the absence of adequate toxicokinetic data to model the range of differences among humans, such differences must be accounted for by including a default toxicokinetic component in the UF_H. The default toxicodynamic portion of the UF_H accounts for differences in target tissue or receptor-mediated response across humans. As noted above, there is limited evidence (Cholakis et al. 1980; Angerhofer et al., 1986) that pregnant rats may be more sensitive to RDX than non-pregnant rats. The intraspecies UF is meant to account for differences across the human population. Pregnant animals represent one potential sensitive subpopulation. For reasons stated above, the SAB agrees the use of a UF_H of 10 is scientifically supported and clearly described.

Key Recommendations

- Consistent with EPA guidance for UFs, the SAB strongly suggests applying the full default UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe neurological effects resulting in use of a severe effect (convulsions) as a basis for the RfD, and the proximity of lethal doses to convulsive doses.
- EPA should discuss whether potential neurodevelopmental effects of RDX would be sufficiently addressed by the default UF_D of 10, given that the mechanism of RDX argues there would likely be developmental neurotoxic effects and the other database uncertainties (lethality at convulsive doses, other less severe neurotoxic effects that may have a lower LOAEL) that also need to be addressed by the UF_D.
- SAB recommends that EPA reconsider the UF for subchronic to chronic extrapolation, and at a minimum, provide stronger justification for a UF_S of 1.

3.3.1.5.Nervous System-specific Reference Dose

Charge Question 3a(v). Is the organ/system- specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

Regarding the RfD for nervous system effects, the POD derived from the neurotoxicity assessment, based on convulsions as the critical endpoint, does not capture all potential adverse outcomes or their severity. This is one reason the SAB recommends increasing the UF_D to 10 (see Section 3.3.1.4). Recognizing the study quality concerns in Cholakis et al. (1980), particularly with respect to the accuracy of administered doses, the EPA assessment should clarify the rationale for utilizing the dose-response data of Crouse et al. (2006) in preference to Cholakis et al. (1980) as the primary basis for the RfD. The POD from the observations in the Crouse study (see

response to charge question 4a) is considered to be more reliable and accordingly should be given more weight.

Overall, the conclusion that the available data in humans and animals support a convulsant neurotoxicity effect for RDX, possibly through a $GABA_AR$ blocking mode of action, is supported scientifically. The proposed nervous system-specific reference dose was clearly described. The SAB supports the derivation of a RfD based on neurotoxicity, but the SAB concludes the scientific support for the methods used to derive the proposed oral RfD is somewhat lacking, primarily due to concerns with the choice of BMR (Section 3.3.1.3) and the value of the database uncertainty factor and the uncertainty factor for subchronic to chronic extrapolation (Section 3.3.1.4).

Key Recommendations

- EPA should justify the rationale for utilizing the dose-response data of Crouse et al. (2006) in preference to Cholakis et al. (1980) as the primary basis for the RfD.
- The SAB recommends increasing the UF_D from 3 to 10.
- The SAB recommends revisiting the UF_S and providing a better justification, at a minimum, for the use of a UF_S of 1.

3.3.2.Kidney and Other Urogenital System Effects

3.3.2.1.Kidney and Other Urogenital System Hazard (Sections 1.2.2, 1.3.1)
Charge Question 3b(i). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

Available Human, Animal, and Mechanistic Studies:
The available human, animal, and mechanistic studies support the conclusion that toxicity to the kidney and other components of the urogenital system is a potential human hazard of RDX exposure. However, this conclusion is primarily supported by animal data, with sparse human studies implicating the kidney as a potential target of RDX that describe transient renal effects following acute human exposure. There are no reports of prostatic effects of RDX in humans and no pertinent mechanistic data regarding RDX effects on the kidney and urogenital system.

Hazards to Kidney and Urogenital System:
All hazards to the kidney and urogenital system are adequately assessed and described in the draft assessment, with the exception of the description of inflammatory changes in the rat prostate. The description in the draft assessment of these prostatic inflammatory changes should include not only suppurative inflammation, but also chronic inflammation and the variability and uncertainty in the classification of prostatic inflammation.

Selection of Suppurative Prostatitis Endpoint:

The selection of suppurative prostatitis as the endpoint ("surrogate marker") to represent renal toxicity hazard is clearly described in the draft assessment, but not scientifically supported because of various uncertainties that are associated with the hazard, including the following:

- There is no known biological or mechanistic basis for using suppurative prostatitis as a surrogate marker for renal and other urogenital (GU) effects.
- There is uncertainty about the direct association between suppurative prostatitis and the toxic renal effects observed in male rats in the Levine et al. (1983) study. A strong association between kidney lesions (papillary necrosis, pyelonephritis, and peri-renal peritonitis) and suppurative inflammation in the prostate was observed only in male rats at the highest dose group (40 mg/kg-day); there were no such renal changes in the lower dose groups (except in one male animal in the 8.0 mg/kg-day group), while suppurative prostatitis occurred at the two next highest doses (8.0 and 1.5 mg/kg-day). The renal lesions were considered primary effects of RDX in the draft assessment, while the prostatitis was considered secondary to the renal effects in terms of severity; the SAB concurs with this notion.
- There are uncertainties regarding the diagnosis of suppurative inflammation:

  (a) Suppurative and chronic inflammation are part of a continuum, and diagnostic criteria may have varied over time and among pathologists. Prostatic inflammation found in aged rats is divided into several subtypes, only one of which is suppurative inflammation. Other categories include subacute inflammation, chronic-active inflammation, and microabscesses. Reference is made in the draft assessment to a paper by Suwa et al. (2001) on the background pathology in the prostate of 1,768 control F344 rats allowed to live for up to 2.4 years. This paper was the basis for the conclusion by EPA that inflammation in the control group of the study by Levine et al. (1983) was unusually low for this strain of rat. However, in the paper by Suwa et al. (2001), all types of inflammation are combined, and 70.4% of these rats had inflammation mostly confined to the dorsolateral prostate and graded as mild. No data were provided by Suwa et al. on suppurative inflammation.

  (b) Combining all types of prostate inflammation in the 24-month groups of the Levine et al. (1983) study yields similar incidences among all groups, with the exception of the highest dose group. The prostatitis incidences in the control and the three lowest dose groups were about 40% lower than the incidences reported by Suwa et al. (2001) for aged F344 rats in NTP studies; the lower incidences may be a reflection of the manner of histopathologic examination (see point c below).

  By contrast, 51 of 55 rats in the high dose (40 mg/kg-day) group of the 24-month study died before the end of the two-year study and 39 of the 51 rats (76%) that died had prostatic inflammation. Twenty-one of the 31 rats (68%) that died after the 12-month timepoint (including the four that survived until the end of study) had prostatic inflammation, which was suppurative in nature in 19 rats and of the chronic type in two rats.

  In the Levine et al. (1983) study, there was a shift from chronic inflammation to suppurative inflammation in the prostate with increasing RDX doses beginning at 1.5 mg/kg-day. This shift is statistically significant if tested using a Chi-squared test, with categories set for no lesions, chronic inflammation, and suppurative inflammation across all treatment groups ($P < 0.0001$); prostatic inflammation was scored by Levine et al. as either chronic

or suppurative. The shift was almost complete in the 31 rats that died in the 40 mg/kg-day group after 12 months on study, as only two animals had (minimal) chronic inflammation and 18 had suppurative inflammation. Although this analysis ideally should have taken into account mortality differences, the Levine study does not contain data that allows one to do this, as pointed out in the draft assessment.

(c) The description of the methods used for histopathological evaluations lacked detail in Levine et al. (1983), which is an important issue given the large variation known for inflammation among the four prostate lobes, based on NTP data of aged F344 rats. The fact that the incidence of prostatic inflammation in the control group of the Levine *et al*. study was 40% lower than the range of inflammation incidences found in the dorsolateral prostate by Suwa et al. (2001) would suggest that some, or many, of the prostates examined by Levine et al. were ventral lobes, which have a low inflammation incidence (4-12%), according to Suwa et al. (2001). Suwa et al. indicated that there was considerable variation in which lobes were present and examined in the NTP studies they reviewed, suggesting that some of the study-to-study variation in the incidence of prostatic inflammation may be due to variations in the prostate lobes examined.

(d) There was no peer review or pathology working group review of the Levine et al. (1983) renal, bladder, and prostate pathology data, as was done for the liver lesions in female mice in Lish et al. (1984).

(e) There may have been potential effects secondary to the high prevalence of fighting among male rats in the highest dose (40 mg/kg-day) group and the resultant individual housing of these animals in the Levine et al. (1983) study. There is evidence in the literature that fighting may cause urogenital infections in male rats (Creasy et al. 2012). Thus, all males in the highest dose group were individually housed from 30-40 weeks into the study, which introduced a significant difference compared to the other treatment groups that may have affected the animals in the 40 mg/kg-day group in uncontrolled ways.

In conclusion, the SAB found that the weight-of-evidence for identifying the prostate as a hazard of RDX exposure is sufficient because: (1) the Levine et al. (1983) study was considered sufficiently rigorous and appropriate for the time in which it was conducted, and adequate to support the conclusion, even though the study has some deficiencies compared to current standards, and (2) the effects on the prostate were dose-related and statistically significant, albeit limited to the rat. The prostatic endpoint of all types of inflammation combined was not changed with increasing RDX dose, except at the highest dose (40 mg/kg-day), where its incidence was significantly increased. Only the incidence of suppurative inflammation and the shift from chronic to suppurative prostatitis were significantly increased at lower dose levels (1.5 mg/kg-day and higher). Both of the latter endpoints would be appropriate for analysis, but the SAB agrees that the suppurative prostatitis incidence data is the most appropriate endpoint for quantitative risk assessment based on dose-response data.

Key Recommendations

- Suppurative prostatitis should not be used as a surrogate marker of renal and urogenital effects, and instead, be considered a separate hazard of RDX exposure (see also Section 3.3.2.5.) for quantitative risk assessment.
- The description and analysis of prostatitis should be expanded to include discussion of both chronic and suppurative inflammation.
- The description of the various uncertainties regarding the Levine et al. (1983) rat study should be expanded to include commentary on the lack of detail on methods used in histopathological evaluations, lack of peer review, and the impact of the high prevalence of fighting in highest dose rats.

3.3.2.2.Kidney and other urogenital system-specific toxicity values (Section 2.1.1).
Charge Question 3.b(ii). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

The selection of the Levine et al. (1983) study that found kidney and other urogenital system effects is clearly described, but not entirely supported scientifically.

While the renal and bladder effects found in male rats in the high dose group of the study by Levine et al. (1983) were treatment-related and the most likely cause of mortality in this group, the effects on the prostate were less straightforward [see also response to charge question 3b(v)].

One male in the lowest dose group (0.3 mg/kg-day) of 55 rats had renal papillary necrosis, but no other animals in the control or 1.5 and 8.0 mg/kg-day RDX dose groups had this lesion. By contrast, renal papillary necrosis was found in 33 of 50 male animals in the high dose group (40 mg/kg-day). Hemorrhagic/suppurative cystitis was found in 35 of 50 male rats of the high dose group, but in only one or two males per group in the lower dose groups and none of the controls. These renal and bladder lesions tended to be more severe after 12 months of study than in rats examined at the six- and 12-month interim necropsies. Prostatic effects, namely a significant shift from chronic to suppurative inflammation, were seen at doses of 1.5 mg/kg-day and above and the overall incidence of prostatic inflammation was significantly increased in male rats in the high dose group (40 mg/kg-day).

The EPA should improve the draft assessment's description and analysis of renal effects observed in studies other than those reported by Levine et al. The Levine et al. (1983) study was not the only animal study that found effects on the kidney. Renal medullary mineralization was reported by Martin and Hart (1974) in three of four males and three of four female Cynomolgus monkeys in the highest dose group tested (10 mg/kg-day), but not at lower RDX doses or in controls. Cortical tubular nephrosis was found in four of ten males and one of ten female B6C3F1 mice at a very high RDX dose of 320 mg/kg-day, while this lesion was not present in control male or female mice (Cholakis et al. 1980). Both studies were of 90-day duration and the apparent renal effects were minimal to moderate in severity and not, or only marginally statistically significant. Cholakis et al. (1980) did not find any renal lesions in male F344 rats and only minimal microcalculi (mineralization) in one of ten female rats exposed to 40 mg/kg-day RDX via the diet for 90 days. In a two-generation study by Cholakis et al. (1980), renal cortical cysts, but no other renal lesions, were found in both control and treated CD (Sprague Dawley) rats.

Another 90-day study in F344 rats used lower doses by gavage and found no evidence of any treatment-related renal effects in males, while minimal-to-mild microconcretions (mineralization) were found in four of ten females that were administered RDX at a dose of 15 mg/kg-day and in seven of ten control females (Crouse et al. 2006). Levine et al. (1981a) found frequent microconcretions (mineralization) in female, but not in male, F344 rats, administered RDX via the diet for 90 days; control rats were without evidence of a treatment related effect. Levine et al. (1981a) also found nephropathy in both sexes, the incidence of which was reduced in the highest dose group (100 mg/kg-day); this reduction was significant in males but not in females. No renal toxicity was found in a 90-day dog study with dietary RDX doses up to 10 mg/kg-day (Hart et al. 1974). The only report of a lesion in the prostate came from the 90-day study by Crouse et al. (2006) in F344 rats administered RDX by gavage at a dose of 15 mg/kg-day; one of eight males had mild subacute inflammation in the prostate, while no prostate lesions were found in ten controls. There were no prostate lesions in any of the other 90-day studies mentioned above. In the 24-month study by Lish et al. (1984), a high frequency of cytoplasmic vacuoles in the renal tubular epithelium, with minimal-to-mild severity, was observed in male B6C3F1 mice at the six, 12, and 24-month time points; the male control group was an exception with only a 10% incidence of these cytoplasmic vacuoles at the six-month interim time point. Female mice had a low incidence of this renal change and this alteration was not reported in any of the other studies mentioned above.

In aggregate, mild toxic effects of RDX exposure on the kidney were found in some species, but not others, and in some studies in both sexes but in other studies only in male or female animals. Of note, some of these effects (mineralization) occurred in a small study with non-human primates, whereas some rodent studies did not find evidence of renal toxicity. Only in the chronic study of Levine et al. (1983) were severe toxic effects on the kidney found and they only occurred in males at the highest dose (40 mg/kg-day); bladder toxicity also occurred in this treatment group, whereas effects on the prostate occurred at doses of 1.5 mg/kg-day and above.

The marked sex difference in the renal toxicity due to RDX exposure found in rats by Levine et al. (1983) is not discussed in the draft assessment. However, there is precedent for a toxic chemical causing renal papillary necrosis selectively in male, but not female, F344 rats (Neal et al. 2003) and several drugs are well known for sex-specificity in their ability to cause renal papillary necrosis (Bach and Nguyen, 1998; Brix, 2002).

Key Recommendations

- Improve the discussion and analysis of renal effects observed in studies other than those reported by Levine et al. (1983).
- Include a brief discussion of the marked sex difference in the renal toxicity in rats due to RDX exposure.

3.3.2.3.Points of Departure for Kidney and Other Urogenital System Endpoints (Section 2.1.2)

Charge Question 3b(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

The SAB strongly recommends that suppurative prostatitis not be regarded as a surrogate marker for kidney and other urogenital system endpoints because of the various uncertainties that are associated with the hazard [see responses to charge questions 3b(i) and 3b(ii)]. If suppurative prostatitis is considered as a stand-alone endpoint (as recommended by the SAB), separate from kidney and other urogenital system endpoints, the calculation of both the POD and HED are scientifically supported and clearly described.

EPA's BMDS software was used to fit ten dose-response models to the data from Levine et al. (1983), and all models provided reasonable fits according to standard goodness-of-fit measures. Using a BMR of 10%, corresponding estimated BMDs for the models ranged from 1.67 to 10.8 mg/kg-day, with associated BMDLs ranging from 0.469 to 8.58 mg/kg-day. BMDLs from the ten models differ by more than threefold, so the lowest BMDL was selected, consistent with EPA guidance. The selected log-probit model has an estimated BMD of 1.67 mg/kg-day, which is within the range of study doses, thus obviating any issues of inappropriate extrapolation. The suppurative prostatitis POD for rats was determined to be 0.469 mg/kg-day.

Three methods were used to calculate the HED corresponding to the BMDL— one based on allometric scaling ($BW^{3/4}$), another based on equivalent RDX serum AUCs in rats and humans at steady state, and a third based on equivalent RDX maximum serum concentrations in rats and humans after dosing. The methods for these calculations are clearly explained. The quality of data used for PBPK modeling is variable with respect to toxicity, but the resulting HED appears appropriate, with preference given to the AUC-based derivation. The SAB finds that the alternative approach of allometric scaling would introduce too many uncertainties.

Key Recommendation

- The SAB strongly recommends that suppurative prostatitis be used as a stand-alone endpoint, separate from kidney and other urogenital system endpoints for calculation of the POD and HED.

3.3.2.4.Uncertainty Factors for Kidney and Other Urogenital System Endpoints

Charge Question 3b(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

The draft assessment used suppurative prostatitis in a two-year study in male rats (Levine et al. 1983) as a surrogate marker for the entirety of observed adverse effects of RDX exposure on the kidney and urogenital system. BMDS models were used to fit the data from Levine et al. (1983) using a 10% benchmark response rate (BMR). The human equivalent dose (HED) for the POD was calculated based on three methods. UFs were then applied to the BMDL10 HED to derive an RfD specifically for the kidney and urogenital system.

The SAB recommends that separate RfDs be derived for the kidney and urogenital system and suppurative prostatis, based on findings of renal papillary necrosis and associated renal

inflammation and prostate effects, respectively. This distinction designates the male accessory sex glands as a separate organ system, and challenges EPA's selection of suppurative prostatitis as a surrogate marker for the adverse effects on the kidney and urogenital system. This recommendation is in keeping with the fact that there is no known mechanistic link between suppurative prostatitis and renal papillary necrosis or adverse effects on renal function. Therefore, the charge question regarding the application of UFs can only be answered at this time for suppurative prostatitis, since a separate RfD has not been derived for renal papillary necrosis. Thus, the comments on the application of UFs are only relevant for the RfD derived based on suppurative prostatitis.

Intraspecies Uncertainty Factor (UF_H)
EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population, which is standard default risk assessment practice. The SAB agrees with the use of a UF_H of 10. (See response to the UF_H response under Section 3.3.1.4 of this SAB report).

Interspecies Uncertainty Factor (UF_A)
An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to the point of departure to account for the remaining toxicodynamic and residual toxicokinetic uncertainty not accounted for in the toxicokinetic modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive a human equivalent dose, and available data are not sufficient to define quantitative toxicodynamic differences between species. The SAB agrees with the application of a UF_A of 3.

Subchronic to Chronic Uncertainty Factor (UF_S)
The draft assessment used a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The Levine et al. (1983) study was a chronic duration exposure study, and thus no extrapolation factor is needed. The SAB agrees that this is appropriate.

LOAEL to NOAEL Uncertainty Factor (UF_L)
The UF_L is meant to account for uncertainties in extrapolating from a LOAEL to a NOAEL when estimating an RfD. A UF_L of 1 was applied because the BMDL was used as a point of departure. Thus, there is no need to extrapolate from a LOAEL to estimate a NOAEL. This is standard risk assessment practice, and the SAB agrees that this is appropriate.

Database Uncertainty Factor (UF_D)
The assessment applied a UF_D of 3 in developing an RfD for suppurative prostatitis. The draft assessment notes that additional studies on neurotoxicity may provide a more sensitive endpoint to use as the basis of an RfD. Thus, a UF_D of 3 was applied across all PODs, regardless of endpoint. In evaluating the RfD based on neurotoxicity, the SAB strongly recommends using a UF_D of 10 rather than 3 due to database limitations. This UF_D would be relevant to an overall RfD based on suppurative prostatitis, if such an RfD were to be the basis of the overall RfD. However, if the RfD for suppurative prostatitis was only to be used specifically in a hazard index approach for this target, then an organ-specific UF_D may be appropriate.

Key Recommendations

- Develop or cite documentation for the use of organ-specific reference values for individual chemicals, including how these would be used in assessing the combined noncancer health impacts of multiple agents acting at a common site in cumulative risk assessments.
- A separate RfD should be derived for renal papillary necrosis and the associated renal inflammation of the kidney and urogenital system.
- The male accessory sex glands should be designated as a separate organ system with a separate RfD derived for suppurative prostatitis.

3.3.2.5. ***Kidney and other urogenital system-specific reference dose*** *(Section 2.1.4).*
Charge Question 3.b.v. Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

The selection of suppurative inflammation of the prostate observed in the Levine *et al*. (1983) study in the draft assessment as a "surrogate marker" of the observed renal and urogenital system effects is not justified [see response to Charge Question 3b(i)] for derivation of a system-specific reference dose (RfD). Therefore, the organ/system-specific reference dose derived for kidney and other urogenital system effects is not sufficiently supported scientifically or clearly characterized.

Key Recommendations
- Separate RfDs should be derived for renal papillary necrosis and the associated renal inflammation and for suppurative prostatitis.
- Available data are not consistent enough across species, doses, sex, or time points to recommend separate candidate RfDs for other, milder renal effects (tubular nephrosis, epithelial vacuolization, and mineralization) found in subchronic studies in mice, rats, and monkeys.

3.3.3.Developmental and Reproductive System Effects

3.3.3.1. Developmental and Reproductive System Hazard
Charge Question 3c(i). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

The SAB's response to Charge Question 3c(i) is subdivided into three components:

<u>*No Evidence of Male Reproductive Effects*</u>
The SAB disagrees with the conclusion in the draft assessment that there is suggestive evidence of male reproductive effects associated with RDX exposure. As discussed in Section 3.3.3.2, the available animal evidence does not support this statement. In addition, several animal studies did not find effects on the male reproductive system. There is no human evidence indicating male reproductive toxicity; no human studies have focused on this question and there were no incidental reports of reproductive effects following RDX exposures. Furthermore, the mechanisms of action of RDX do not provide reasons to expect male reproductive toxicity.

Conclusions as to whether Developmental Effects are a Human Hazard of RDX Exposure
The SAB concludes that there are sufficient available data to draw the conclusion that RDX does not pose a risk of induction of structural malformations during human fetal development based on studies in rats and rabbits at doses that were high enough to occasionally produce maternal toxicity. Additionally, the SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity (decreased fetal weight, increased post-implantation loss), as these effects occurred only at maternally toxic dose levels.

The developmental toxicity observed was typical of findings associated with maternal toxicity and occurred at maternally toxic dose levels. It is generally understood that maternal toxicity, evidenced by body weight loss or reductions in body weight gain and/or decreases in food consumption, can contribute to developmental toxicity of the fetus in animal models. Developmental toxicity associated with maternal toxicity typically manifests as fetal weight reductions, increases in post-implantation loss (i.e., embryo/fetal death), and increases in the incidence of certain fetal skeletal variations. There is recognition within the scientific community of the possible effects on the fetus from maternal toxicity in common animal models [Carney and Kimmel, 2007; Rogers et al. 2005]. This concept was the primary topic discussed in an International Life Sciences Institute-Health and Environmental Sciences Institute (ILSI-HESI) sponsored working group, and the proceedings have been published [Beyer et al. 2011]. The findings in the RDX developmental toxicity studies of increased post-implantation loss, decreased fetal body weight and fetal skeletal variations, are those considered typically associated with maternal toxicity and occurred at maternal toxic dose levels.

In an embryo fetal developmental (EFD) toxicity study in F344 rats, maternal toxicity (mortality up to 31%) and developmental toxicity (reduced fetal body weights and increased resorptions) occurred at 20 mg/kg-day (Cholakis et al. 1980). In Sprague Dawley rats administered 20 mg/kg-day RDX via gavage, maternal toxicity and increased resorptions were observed (Angerhofer et al. 1986). No structural malformations occurred at these doses or lower doses in either rat strain. Treatment in both of these studies starts on gestation day 6, while implantation is still in progress and ends on gestation day 15, prior to the closure of the hard palate. A longer dosage period as suggested for all current EPA (U.S. EPA, 1998a) and OECD (2015) guidelines, may have yielded more fetal toxicity, especially an effect on fetal weight.

The only two-generational study identified in the literature reported decreased offspring survival (including stillborn pups and postnatal deaths through the age of weaning) following a clearly maternally toxic dose of 50 mg/kg-day that was administered in the diet and adjusted approximately weekly (Cholakis et al. 1980). Lower doses were not toxic to the dams or offspring.

Rabbits evaluated in an embryo-fetal developmental toxicity study dosed on days 6 to 29 of gestation appear to be less sensitive than rats as exposures up to 20 mg/kg-day did not produce any maternal or embryo/fetal toxicity (Cholakis et al. 1980). The rabbit embryo fetal development study at 0.2, 2 and 20 mg/kg-day showed fetal malformations with a low incidence at the 20 mg/kg-day dose and these changes were not present in control fetuses or seen at lower dose levels. The incidences ranged from 1 to 3 % and included a variety of malformations with no apparent biological relatedness, none of which were statistically significant. These data are difficult to put in context without a robust historical control database, systemic exposure levels and

knowledge of the litter size. The report states that the findings were not statistically significant and thus not attributed to RDX exposure in rabbits.

Other Hazards to Human Reproductive and Developmental Outcome
Based on *in vitro* data, the SAB concludes that the mechanistic-based hazard demonstrating RDX inhibits GABAergic neurons presents a potential neurodevelopmental hazard that was not adequately addressed in the draft assessment. Several lines of evidence point to potential window(s) of susceptibility in the developing brain to chemicals interfering with GABAergic systems (see discussions in Sections 3.3.1.1, 3.3.1.2, and UF_D discussion in Section 3.3.1.4).

A pilot developmental neurotoxicity study was conducted in rats that demonstrated significant accumulation of RDX in the immature brain of offspring and in the milk from dams treated with 6 mg/kg-day during gestation (Hess-Ruth, 2007). This dose level induced convulsions in adult animals. There were approximately equal concentrations (µg/mL) in maternal blood and milk, and higher levels in younger postnatal day (PND) 1 pup brains compared to PND 10. A stated conclusion from this report was that further studies evaluating neurotoxicity and developmental effects of RDX should be conducted. It does not appear that a follow up study was conducted, thus no definitive assessment of potential developmental neurotoxicity in rats can be completed to inform risk for humans. Regardless, the SAB encourages the inclusion of a description of the potential mechanistic-based hazard in the draft assessment based on the reported mechanism to inhibit GABAergic neurons and the knowledge that RDX is present in the brain of developing rats during gestation and lactation.

Key Recommendations
- Due to significant weaknesses of the findings in the rat and mouse studies, RDX should not be considered as having suggestive evidence of male reproductive effects.

3.3.3.2.Reproductive System-Specific Toxicity Values
Charge Question 3c(ii). Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?

In consideration of all evidence, the SAB does not agree that the selection of Lish et al. (1984) for male reproductive effects is supported scientifically, and offers further suggestions on how to describe these data.

The SAB finds that the suggestive evidence of male reproductive effects provided by Lish et al. (1984), based on testicular degeneration in male mice exposed to RDX in their diet for 24 months, is weak, unsupported by other endpoints in that study showing no effect, complicated by the age of the mice and general toxicity of the RDX dose, and contradicted by most other studies.

In the study of Lish et al. (1984), the 10% and 11% incidence of testicular degeneration observed at doses of 35 and 108 mg/kg-day was not considered to be statistically significant. Also, no histological changes were observed at six or 12 months of study, durations that were much longer than the 1.4-month duration of spermatogenesis in mice. Furthermore, significant decreases in testis weight, which should have been observed if there were appreciable degeneration, were not observed.

The validity of 24-month chronic toxicity studies to evaluate male reproductive toxicity in rodents is questionable because of the loss of testicular function that occurs with aging in both rats and mice. In rats, the manifestations of aging in 2-year old animals include a high incidence of interstitial cell tumors (Cohen et al. 1978), declines in sperm production (Wang et al. 1993; Johnson & Neaves, 1983), reduced gonadotropin levels (Bruni et al. 1977), and reduced testosterone production due to aging of Leydig cells (Chen et al. 2002). In 2-year old mice, reductions in sperm counts and hormone levels were also observed (Bronson & Desjardins, 1977; Gosden et al. 1982), along with reductions in the numbers of stem spermatogonia, the loss of functional ability of theses stem cells, and failure of the somatic environment to support spermatogonial differentiation (Suzuki & Withers, 1978; Zhang et al. 2006). Effects observed in rodents exposed to a potential reproductive toxicant in a 2-year chronic toxicity study might represent the combined effects of toxicant and aging, and not the result of prolonged treatment.

In addition, the indication of an effect of RDX on spermatogenesis suggested by Lish et al. (1984) is generally not supported by other studies (Table 3). In particular, Cholakis et al. (1980), using the same mouse strain, did not find any significant effects of RDX doses up to 320 mg/kg-day in a 3-month subchronic study. Although the RDX used by Cholakis et al. was of larger particle size than that used by Lish et al. which could reduce the uptake of RDX, mortality of the animals in the Cholakis et al. study administered 320 mg/kg-day was equivalent to that observed by Lish et al. at 175 mg/kg-day, indicating effective uptake of the RDX particles. Since 3-months allows for more than two complete rounds of spermatogenic cell differentiation, this should have been sufficient time to detect a toxic effect.

Furthermore, studies in rats indicate little male reproductive toxicity of RDX. In a 2-year chronic study, Hart et al. (1976) found no testicular degeneration or weight loss at doses up to 10 mg/kg-day. Similarly, Levine et al. (1983) found no effects of a dose of 8 mg/kg-day. However, at 40 mg/kg-day there was a significant decline in testis weight (14%) and a significant increase in the percentage of testes showing germ cell degeneration at 12 months of treatment. Although the effect was significant, the fact that there was 30% excess mortality by this time may indicate that the testicular damage was secondary to general toxicity. Data obtained at 24 months were not meaningful since all rats of this strain developed Leydig cell hyperplasia/neoplasms by 2 years of age.

Three 13-week subchronic studies in rats also failed to indicate significant testicular damage. Levine et al. (1981a, b; 1990) found no significant testicular effects of exposure at doses up to 100 mg/kg-day. Also, Cholakis et al. (1980) found no changes in absolute testis weights or histopathological damage to testes at 28 or 40 mg/kg-day. Similarly, Crouse et al. (2006), in the only study using gavage, which had greater potency than dietary administration as indicated by 20-30% mortality at doses of 10-15 mg/kg-day, reported no significant histological effects in testes or changes in absolute testis weights. The additional data of Cholakis et al. (1980) obtained as part of a 2-generational study, did indicate an 18% reduction in proportions of females impregnated by males exposed to RDX at 50 mg/kg-day. While this could reflect a testicular effect, it could also be a behavioral effect or a systemic effect, as suggested by the 14% excess mortality in this group.

Table 3. Summary of Results of 7 Studies of Male Reproductive Toxicity of RDX

Study	**Species**	**Route**	**Significant Effect**	**Doses** (mg/kg-day) **Time** (months)	**Caveats**	**Negative Results** (non-significant considered as negative)
Lish et al. (1984)	Mouse	Diet	None	35 & 108 24 mo.	Mortality* (>14%) Age-related effect	No histological change at 6 or 12 mo. The 10-11% incidence in testicular degeneration at 24 mo. was not significant. No decrease in testis weight
Cholakis et al. (1980)	Mouse	Diet	None	40, 80, 160, 320 3 mo.		No histological changes No decrease in testis weight
Levine et al. (1983)	Rat	Diet	40% increase in incidence of germ cell degeneration 14% decrease in testis weight	40 mg/kg-day 12 mo.	Mortality* 31% at 12 mo.	No effects at 8 mg/kg-day No effects at 6 months with 40 mg/kg-day No germ cell degeneration at 40 mg/kg-day at 24 mo.
Hart (1976)	Rat	Diet	None	10 mg/kg-day 12 & 24 mo.		No histological changes (24 months) No decrease in testis weight
Cholakis et al. (1980)	Rat	Diet	18% reduction in proportion of females impregnated †	50 mg/kg-day 3 mo.	Mortality* 14% Possible behavioral effect	Reduction in impregnation not observed at 16 mg/kg-day No histological changes at 40 mg/kg-day No decreases in testis weight at 28 or 40 mg/kg-day
Levine (1981a,b; 1990)	Rat	Diet	None	10, 30, 100 3 mo.		No histological changes No decreases in testis weight
Crouse et al. (2006)	Rat	Gavage	None	15 mg/kg-day 3 mo.		No histological changes No decreases in testis weight

* Excess mortality compared to observed in controls

† Calculated as significant by reviewer using Chi-square test at P=0.004

Finally, the SAB did not find the selection of Lish et al. (1984) to be clearly described, and provided specific comments in Appendix B on the text, tables and figures to improve presentation of data on reproductive and developmental toxicity.

Key Recommendation

- SAB finds that derivation of a reproductive-system specific toxicity value is not justified, as there have been no convincing studies showing significant male reproductive toxicity.

3.3.3.3.Points of Departure for Reproductive System Endpoints

Charge Question 3c(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

As discussed in Sections 3.3.3.1 and 3.3.3.2, the SAB does not support use of the Lish et al. (1984) study for describing male reproductive system effects, because the suggestive evidence of male reproductive effects provided by Lish et al. (1984) is weak, unsupported by other endpoints in that study showing no effect, complicated by the age of the mice and general toxicity of the RDX dose, and contradicted by most other studies. Given that Lish et al. (1984) was the data source for dose-response modeling and subsequent derivations of the POD and HED, these derived POD and HED are not scientifically supportable.

Key Recommendation

- No POD for reproductive endpoints should be calculated from the existing data and therefore there is no need to calculate the HED.

3.3.3.4.Uncertainty Factors for Reproductive System Endpoints.

Charge Question 3c(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

The draft assessment used the data on testicular degeneration in mice from a 2-year dietary study (Lish et al. 1984) as the basis for derivation of the POD. BMDS models were used to fit the incidence data to derive a BMDL for a 10% BMR. Three methods were used to derive an HED from the mouse POD. The draft assessment notes that the toxicokinetic data available for the mouse are not as robust as for the rat, and thus confidence in the use of PBPK modeling to account for interspecies toxicokinetics is low. Rather, the default allometric scaling approach was used to derive the HED by scaling dose by ¾ power of body weight. After adjusting the mouse POD to an HED with this scaling, UFs were applied to derive an RfD for male reproductive toxicity.

The SAB does not support derivation of an RfD based on male reproductive system effect [see Section 3.3.3.3], and concludes that an RfD based on testicular degeneration is not supported scientifically. The question of UFs as applied to the POD is therefore extraneous.

Key Recommendation

- Since no valid POD should be calculated for reproductive endpoints, there is no need to discuss UFs for reproductive endpoints.

3.3.3.5.Reproductive System-specific Reference Dose.

Charge Question 3c(v). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

The RfD for reproductive effects based on testicular degeneration is clearly described but not scientifically supported. Reasons for this conclusion are provided above in Section 3.3.3.2, and briefly summarized below.

Testicular degeneration was reported at terminal sacrifice (24 months) in one 2-year dietary study in mice (Lish et al. 1984). Germ cell degeneration was also observed in a 2-year dietary study in rats (Levine et al. 1983) but only at the 12-month interim sacrifice and not at the 6-month interim or 24-month terminal sacrifice. The SAB notes that testicular histopathology should have been seen at earlier time points (e.g., the 6-month and 12-month interim sacrifices) in Lish et al. (1984), as these exposure durations were several times longer than the 1.4-month duration of spermatogenesis in mice. Further testicular degeneration was not observed in the majority of the dietary and gavage studies in rodents (5 of 7 showed no effect).

Other reproductive effects observed included changes in testicular absolute and relative weights, but these findings were inconsistent across studies. Effects on fertility were noted in a 2-generation reproductive study in CD rats at the high dose (50 mg/kg-day) (Cholakis et al, 1980), but both the male and female rats had decreased weight gain and increased mortality. Thus, it was difficult to attribute the reduction in fertility to a specific reproductive toxicity effect of RDX. In a dominant lethal assay (Chokakis et al. 1980), the decreased rates of pregnancy of untreated females that were mated with F0 males treated with RDX at 50 mg/kg-day may have been associated with generalized toxicity in the treated males rather than a specific effect of RDX. There were no observations of histological changes in the testis or decreased testicular weight in any of the treated animals in Cholakis et al. (1980).

The EPA provided the BMDS analysis in Appendix D of the Supplemental Document, and clearly described the rationale for deriving the HED and applying the UFs. However, since the toxicological effect used as the basis of the RfD was testicular degeneration, and this is not supported scientifically, then the RfD is not supported scientifically.

Key Recommendation

- No RfD based on male reproductive toxicity should be calculated since no valid POD can be estimated.

3.3.4.Other Noncancer Hazards

Charge Question 3d. The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal and mechanistic studies support this decision. Are other non-cancer hazards adequately described?

<u>Liver, Ocular, Musculoskeletal, Cardiovascular, Immune, and Gastrointestinal Effects</u>

In the process of identifying the health hazards of RDX, a conclusion should be made for each hazard endpoint discussed based on available evidence streams (human, animal, and mechanistic), and a critical evaluation of the quality and relevance of the data reviewed. In this regard, the SAB recommends that the draft assessment be explicit as to whether or not the available evidence supports each of the discussed systemic effects as a potential human hazard, and the rationale for reaching that conclusion. Furthermore, the meaning of the statement (line 11, p. 1-60 and lines 13 & 14, p. 1-69) "at this time no conclusions are drawn regarding [*viz.* liver effects or other non cancer effects] as human hazards of RDX exposure", is not clear. Specifically, the draft assessment should clarify whether existing data are inadequate to establish that RDX can cause a particular adverse effect in humans, or whether existing data are inadequate quantitatively to serve as a POD for a risk assessment. Clearly, very high unspecified doses of RDX cause modest, reversible increases in liver-specific serum enzyme activities in humans. High doses of RDX cause modest increases in serum enzyme activities and hepatomegaly in dogs, and while RDX does not appear to enhance serum enzymes in rats, it produces increased liver weights. However, increased relative liver weights are not observed consistently from one study to another. In light of these observations, simply stating that "no conclusions are drawn regarding liver effects as a human hazard of RDX exposure" leaves the reader uncertain as to what decision EPA has made and why.

The description of Liver Effects in Section 1.2.4 is well written and comprehensive. The authors have done an excellent job grouping studies and providing detailed accounts. Conclusions about consistency of inter- and intra-species findings of different durations are scientifically appropriate. The integration of the liver effects on the top of the page 1-61 should lead to a more specific/definitive conclusion, as noted above, rather than the conclusion stated in lines 10 and 11.

It is recommended that the overviews of ocular, cardiovascular, musculoskeletal, immune, gastrointestinal and hematological effects be moved from Section C.3.2 of Appendix C to a new subsection 1.2.5 (Other Noncancer Effects), rather than be included as an Appendix that readers may not be able to access readily. As such, Section 1.2.5 (Carcinogenicity) would become Section 1.2.6.

The accounts of ocular, cardiovascular system, musculoskeletal system, immune system, gastrointestinal system, and hematological system effects of RDX, like those for the liver, are generally detailed, accurate and comprehensive in their coverage of each organ system. It is laudable that each account, with the exception of the musculoskeletal system, is concluded by a definitive, well-supported summary statement of the available evidence streams. However, these summaries lack a conclusion and rationale for whether the evidence supports potential human hazards from RDX exposure. The following additional information may be helpful in developing conclusions and a related rationale.

It is stated in lines 24-26 of page C-44 that muscle injury was indicated by elevated levels of aspartate aminotransferase (AST) or myoglobinuria. However, some other enzymes measured in serum are more specific for muscle damage. Kucukardali et al. (2003), for example, reported transient increases in several serum enzymes in four of five patients experiencing RDX-induced seizures. One of the enzymes, creatine phosphokinase (CPK), is primarily indicative of muscle damage. Testud et al. (2006) measured elevated CPK and myoglobin levels in an Octogen-

poisoned patient. The clinicians attributed these findings to muscle damage secondary to seizures.

The EPA concludes in lines 5 and 6 of page C-46 that histopathological changes have generally not been reported in RDX dietary studies. Kucukardali et al. (2003) did observe gastroduodenitis by endoscopy in three of five human poisoning victims who ingested enough RDX to cause protracted seizures. Severe irritation of the gastrointestinal mucosa by direct contact with RDX would account for the nausea and vomiting commonly experienced by humans who ingest high doses of the chemical.

With respect to effects of RDX on the immune system, the empirical data have been summarized adequately in Appendix C.3.2. Based on the available animal studies, consistent dose-related immune system effects from oral exposure to RDX were not observed. However, it should be noted that none of these studies, including that of Crouse et al. (2006), completed sensitive immune function evaluations. The Crouse study was specifically designed to evaluate immunotoxicity in rats, but included only less sensitive structural evaluations of the immune system, such as populations of red and white blood cells, proportion of cell surface markers, cellularity in proportion to organ weight, B and T cells in the spleen, and CD4/CD8 antigens of maturing lymphocytes in the thymus). As noted by USEPA (1998), WHO (2012), and others, evaluation of such structural parameters in the absence of more sensitive functional testing is unlikely to detect immunosuppression, unintended immune stimulation, autoimmunity, or dysregulated inflammation.

Other Non-Cancer Hazards:

The potential "other non-cancer hazards" from RDX exposure are identified and discussed in Section 1.2.4 and 1.3.1 (liver), and Section 1.2.6 and Appendix C.3.2 (ocular, musculoskeletal, cardiovascular, immune system, gastrointestinal, and hematological) of the draft assessment. In Appendix C.3.2, lines 5-6 it states "Overall, at the present time, the evidence does not support identifying these other systemic effects as human hazards of RDX exposure." In the subsequent paragraphs summarizing the evidence for the other systemic effects, the text does not provide a clear rationale for why the evidence does not support the listed effects as potential human hazards. Importantly, it should be specified whether the conclusion is due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not sensitive endpoints.

Neuroinflammation has emerged as a key characteristic of most neurological conditions, including seizure and epilepsy, as recently reviewed by Dey et al. (2016) and Eyo et al. (2017). In particular, RDX-induced seizures may trigger acute immune and inflammatory responses within the brain, while chronic neuroinflammation may result from recurrent seizures. Neuroinflammation, in turn, has a proconvulsant effect by lowering the seizure threshold, influencing seizure severity and recurrence. This context is relevant to the interpretation of studies in which RDX exposures provoked convulsions. It is less clear what relationship, if any, there may be between less severe manifestations of RDX neurotoxicity and neuroinflammatory or other chronic immune system responses.

Not addressed in the draft assessment were the dose-related effects on body weights and/or body weight gains, although this was identified as a potential adverse effect of RDX elsewhere (e.g.,

Sweeney et al. 2012a, b; U.S.EPA, 2012b). Dose-related decreases in body weight gain were frequently observed in repeated dose studies with RDX, and should also be considered and discussed as a potential noncancer effect. Reduction in body weight is a common manifestation of adverse effects of chemicals, reflecting generalized systemic toxicity. This parameter has been utilized in numerous IRIS assessments for the derivation of reference values. The RDX literature should be screened to identify subchronic or chronic animal studies in which dose-dependent decreases in body weight/body weight gain are reported. Dose-related body weight effects should be discussed in the draft assessment, including their suitability to carry forward from hazard identification to the dose-response analysis.

Key Recommendations

- For each of the other noncancer hazards discussed in the draft assessment, add a summary statement regarding whether the available studies do, or do not, support a conclusion that the identified toxicity is a potential human hazard. Include an explanation of the rationale for reaching the conclusion, taking into consideration the additional information pertaining to liver effects, the muscle injury, immune system, neuroinflammation and gastrointestinal effects, as detailed above by the SAB.
- Include as a potential noncancer hazard the available subchronic and chronic data on body weight/body weight gain, and whether the studies do, or do not, support a conclusion that body weight effects represent a potential systemic human hazard. Discuss the rationale for the conclusion and explain why body weight effects are or are not carried forward to the dose-response analysis.

Suggested Recommendation

- Move the overviews (and associated tables) of ocular, cardiovascular, musculoskeletal, immune, gastrointestinal and hematological effects from Section C.3.2 of Appendix C to Section 1.2 of the main body of the draft assessment. These overviews should be placed in subsection 1.2.5 (Other Noncancer Effects) rather than be part of an Appendix. Section 1.2.5 (Carcinogenicity) would become Section 1.2.6.

3.3.5. Cancer

3.3.5.1. Cancer Hazard

Charge Question 3e(i). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

The SAB concurs with the EPA that "*suggestive evidence of carcinogenic potential*" is the most appropriate cancer hazard descriptor for RDX and that this descriptor applies to all routes of human exposure

In the draft assessment, the EPA considered two potential hazard descriptors under the EPA's *Guidelines for Carcinogenic Risk Assessment* (USEPA, 2005): "*likely to be carcinogenic to humans*" and "*suggestive evidence of carcinogenic potential*," with the latter indicative of a lesser

weight of evidence. Per established guidelines, the *suggestive evidence of carcinogenic potential* descriptor is "appropriate when the weight of evidence is suggestive of carcinogenicity, a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion." A *likely to be carcinogenic in humans* descriptor is appropriate when "the weight of evidence is adequate to demonstrate carcinogenic potential to humans" but is not strong enough to justify the highest weight of evidence descriptor *carcinogenic in humans*.

The draft assessment noted that RDX tested positive in more than one species, sex, and strain in animal studies, and that this evidence was consistent with a "*likely to be carcinogenic in humans"* descriptor, as provided in the EPA guidelines (USEPA, 2005), and suggested that more than one descriptor might apply to RDX. However, the draft assessment also noted that the evidence of carcinogenicity outside the B6C3F1 mouse was not robust, and this factor was decisive in choosing a hazard descriptor, which was "*suggestive evidence of carcinogenic potential."*

In considering the most appropriate cancer hazard descriptor for RDX, the SAB evaluated the strength of evidence for positive cancer findings. The SAB agrees with the EPA that the relevant observations are the liver tumors that were observed in female B6C3F1 mice and male F344 rats and lung tumors that were observed in female B6C3F1 mice in two-year dietary bioassays (Lish et al. 1984; Levine et al. 1983) and identified other limitations that raised concerns.

The findings of the SAB are as follows:

1) <u>*Mortality in the high dose groups*</u>. The high dose of RDX in the Lish et al. dietary study in mice was initially 175 mg/kg; however, the dose was reduced at week 11 of the study to 100 mg/kg diet, due to acute toxicity and high early mortality (30 of 65 males and 36 of 65 females). The acute toxicity and early mortality reduced the effective number of animals after 11 weeks on the study to 35 male mice and 29 female mice. Twenty-two male and 25 female mice in the high dose group survived to the scheduled sacrifice at 24 months. Similarly, the high dose in the Levine et al. dietary study in rats was 40 mg/kg-day, and mortality was high throughout the study period of 24 months. Unlike the mouse study, mortality occurred gradually over the entire period of the rat study, with mortality in most rats occurring after 6 months. Male rats were particularly affected by RDX toxicity, and histopathological evaluations indicated that the high mortality was likely due to renal disease. Four of 55 males and 28 of 55 females in the 40 mg/kg dietary exposure survived to scheduled sacrifice. The *Guidelines for Carcinogen Risk Assessment* states that "If toxicity or mortality is excessive at the high dose, interpretation [of cancer] depends on whether or not tumors are found. … Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits."

2) <u>*Liver tumors in rats*</u>. A positive finding of cancer hazard in two species is based on the liver tumor response in male F344 rats to RDX, in addition to the liver and lung tumors observed in female RDX-exposed mice. The liver tumor response of males to RDX in the Levine et al. (1983) rat study was only significant in a trend test, if the incidence of hepatocellular carcinomas in males of the high dose group (40 mg/kg-day) was included. When this group was omitted from the analysis due to high mortality, the trend was not significant nor was a pair-wise

comparison of the high dose group incidence to that of control males. There was no dose-related trend for the incidence of adenomas or the combination of adenomas and carcinomas. Although the incidence of benign liver tumors in control males was on the high end of the range for historical controls (Haseman et al. 1985), the evidence for an association of RDX exposure with increased liver tumors in this rat study is weak.

3) *Lung tumors in mice*. The increased incidences of liver and lung tumors observed in female $B6C3F_1$ mice would support a positive finding of cancer hazard at two sites. However, the lung tumor response to RDX in the mouse study of Lish et al. (1984) showed a significant trend for an increase only when the incidence of lung tumors (adenomas and carcinomas combined) in females of the high dose group (175/100 mg/kg-day) was included in the trend test. The trend and pairwise comparison tests were not significant if the high dose group was excluded from the analysis. A positive trend for the incidence of pulmonary carcinomas (not adenomas) was observed in both sexes of mice, but only when the high dose groups were included. The incidence of these tumors was quite close to that observed in historical controls (Haseman et al. 1985). Thus, the evidence for an association of RDX exposure with increased lung tumors in this mouse study is weak and solely driven by the findings in the high dose group that suffered from high early mortality.

4) *Liver tumors in mice*. A positive finding of cancer hazard in both sexes of one species is based on the liver tumor response in male and female $B6C3F_1$ mice to RDX, but the SAB identified several concerns regarding the liver tumors in mice.
   - Although there were suggestive increases in liver tumors in male mice, none of the increases appear to be statistically significant, using either a trend test or pairwise comparison tests. Of note, the incidences of these tumor types are quite variable in mice and the observed increases are within the range of incidences observed in historical controls (Haseman et al. 1985). Of further note is that the incidence of combined adenoma and carcinoma liver tumors observed in the high dose group is near the high end of the historical control range and that increases in tumors at other sites were not observed in male mice.
   - The liver tumor findings were more robust in female mice, but there were also concerns with these observations, due to the unusually low incidence of hepatocellular tumors in female control mice. None of the concurrent female mice controls had hepatocellular carcinomas (0.0%) and one of 65 had a liver adenoma (1.5%), while historical incidence control data published by the NTP were 8.0% (range 0-20%) for the combined hepatocellular carcinoma and/or adenoma, indicating that the observed 1.5% incidence was notably at the low end of the range of incidences found in historical controls.
   - In a reevaluation of hepatocellular neoplasms in female mice by a Pathology Working Group (PWG), the original histological sections from female mice were retrieved and a second examination was performed by pathologists (Parker et al. 2006). It was noted that a reevaluation of neoplasm sections from just one sex is unusual; sections from both male and female animals would be reevaluated typically to ensure that findings in both sexes were reliable. Members of the PWG then reexamined all hepatocellular neoplasm sections from female mice and cited factors that reduced their confidence in a positive interpretation of the study. The cited factors included variations in the number of liver sections per mouse, the absence of precursor lesions, such as foci of cellular alteration, and, most importantly, the low incidence of hepatocellular neoplasms (1.5%) in the control females. A

discrepancy in the number of mice examined by Lish et al. (1984) and by Parker et al. (2006), while not major, further undermined confidence in the quality of the data.

5) *Non-neoplastic histopathological liver changes.* Non-neoplastic histopathological changes in the liver were absent in the majority of subchronic studies available in the literature, and pre-neoplastic lesions were absent in the livers of mice and rats at interim sacrifices conducted at 6 and 12 months in the two-year bioassays by Lish et al. (1984) and Levine et al. (1983). The finding that non-neoplastic changes in livers were not associated with RDX exposure in the majority of animal studies suggested that intrinsic factors may be involved in the observed tumor findings, especially in light of the fact that the mode of action of RDX carcinogenicity cannot be determined based on the current understanding of RDX metabolism (see below). It is acknowledged that the absence of hepatic precursor lesions does not, in itself, negate the possibility that RDX could have caused increases in liver neoplasms. Nevertheless, this is a weight of evidence factor to consider for the carcinogenicity of RDX.

6) *The lack of pathology peer-review and available data to support mortality-based statistics for neoplasms in the two-year bioassays by Lish et al. and Levine et al.* Carcinogenicity findings in well-conducted experimental animal studies are regarded as evidence of potential cancer risk to humans by national and international health agencies. In order for experimental animal studies to serve as reliable sources of data for the evaluation of the carcinogenic potential of environmental agents, certain criteria should be met (Melnick et al. 2008). These include: a) animal models that are sensitive to the end points under investigation; b) detailed characterization of the agent and the administered doses; c) challenging doses and durations of exposure (approximately 2 years for rats and mice); d) sufficient numbers of animals per dose group to be capable of detecting a true effect; e) multiple dose groups to allow characterization of the dose-response relationships; f) complete and peer-reviewed histopathologic evaluations; and g) pairwise comparisons and analyses of trends based on survival-adjusted tumor incidence (Melnick et al. 2008). The Lish et al. and Levine et al. studies met criteria a – e; however, complete and peer-reviewed histopathologic evaluations and pairwise comparisons and analyses of trends based on survival-adjusted tumor incidence were not conducted and the available data did not allow EPA to perform these analyses. Additionally, necropsy and histological processing records were not available to link gross lesions observed at necropsy or the number of gross lesions with histological sections that were evaluated for each animal.

7) *Limited evidence to support a mode of action for RDX carcinogenicity.* Data are not available in the literature to properly evaluate the metabolism of RDX by human liver or lung enzymes or by human microflora to form genotoxic agents. One rodent study demonstrated the reductive transformation of RDX to N-nitroso compounds (Pan et al. 2007b). It is unclear if this transformation occurred via microflora, non-enzymatic processes, or by rodent metabolic enzymes. Bhushan et al. (2003) reported that rabbit cytochrome P4502B4 converts RDX to 4-nitro-2,4-diazabutanal *in vitro*. This compound and 4-nitro-2,4-diaza-butanamide were identified as minor end product metabolites in the urine of Yucatan miniature pigs (Major et al. 2003). However, the genotoxic potential of these compounds has not been determined in mutagenesis assays. Numerous studies have shown that RDX yields negative test results with the Ames *Salmonella typhimurium* assay in a variety of bacterial strains (Cholakis et al. 1980; George et al. 2001; Tan et al. 1992) and is not cytotoxic or mutagenic in the in vitro mouse

lymphoma test or in vivo by the mouse bone marrow micronucleus test (Reddy et al. 2005). RDX was reported by one group to be weakly mutagenic in one strain of Salmonella typhimurium using S9 activation (Pan et al. 2007a) and showed some evidence of mutagenic activity in *Vibrio fischeri* using the Mutatox assay (Arfsten et al. 1994). In vitro biotransformation studies on RDX suggest that RDX can be metabolized by anaerobic bacteria in soils to form N-nitroso derivatives. Such biotransformation has also been demonstrated in the mammalian gastrointestinal tract (Major et al. 2007; Musick et al. 2010; Pan et al. 2007b). These minor N-nitroso metabolites, MNX and TNX, were reported to be positive in *in vitro* genotoxicity studies using several strains of *Salmonella typhimurium* (Pan et al. 2007a; George et al. 2001). Moreover, MNX was reported to be positive in genotoxicity studies in mammalian cells *in vitro* with metabolic activation with S9 (Snodgrass, 1984). Other modes of action of RDX, such as oxidative stress, have not been investigated. However, it should be noted that, while understanding the mode of action can sometimes support a concern for carcinogenicity of a chemical, it is not requisite to the determination of its cancer hazard.

Based on the guidance provided in the EPA's *Guidelines for Carcinogenic Risk Assessment* (USEPA, 2005) and points 2-4 above, the SAB considers that the evidence for a positive tumor response to RDX in two species, two sexes, or two sites required by EPA for a "*likely to be carcinogenic in humans"* descriptor is weak or absent. Given the limitations and nature of the carcinogenicity data available, the SAB concludes that the descriptor, "*suggestive evidence of carcinogenic potential"*, is appropriate. As noted in the draft assessment and in the discussion above, oral exposure to RDX has been observed to result in tumors in liver, which is beyond the point of initial contact. This is indicative of carcinogenic effects that are systemic rather than confined to the portal of entry to the body, and thus carcinogenic potential is independent of the route of exposure. Therefore, the SAB agrees with EPA that this descriptor applies to all routes of exposure.

Key Recommendation

- Strengthen and make more specific the justification for selecting the "*suggestive evidence of carcinogenic potential"* descriptor rather than the *"likely to be carcinogenic to humans"* descriptor.

Suggested Recommendations

- Expand the discussion to include more on the limitations of the Lish et al. (1984) and Levine et al. (1983) animal studies.
    - Clarify that the absence of hepatic precursor lesions in the female mice of the Lish et al. (1984) study does not, by itself, negate the possibility that RDX could have caused the increases in liver neoplasms.
    - Include a more complete description of the differences in mortality time course between mice in the Lish et al. (1984) study and rats in the Levine et al. (1983) study administered the high dose level of RDX in the diet and the potential impact of these differences on the interpretation of the hepatic and pulmonary neoplasms in female mice.

3.3.5.2. Cancer-specific Toxicity Values.

Charge Question 3e(ii). As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response

assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

The SAB finds that the draft assessment adequately explains the rationale for a quantitative analysis of RDX cancer assessment and that the selection of the Lish *et al*. (1984) study for this purpose is supported scientifically and clearly described.

Despite concerns associated with interpretation of the data as discussed in response to Charge Question 3e(i) (Section 3.3.5.1) that raise questions about the suitability of the data for quantitative analysis, the Lish et al. (1984) study met most of the seven criteria used by national and international health agencies in identifying studies to serve as reliable sources of data for evaluation of the carcinogenic potential of environmental agents (see point 6 in Section 3.3.5.1). The study was a well-conducted two-year bioassay that included a large number of animals tested at multiple dose levels, and increased incidences of neoplasms occurred in exposed female mice. Moreover, the hepatocellular neoplasms in female mice in Lish et al. (1984) were reevaluated by a PWG (Parker et al. 2006). The updated liver tumor incidences from the PWG reanalysis of Lish et al. (1984) were used by EPA for quantitative dose-response analysis.

3.3.5.3. Point of Departure (POD) for Cancer Endpoints.
Charge Question 3e(iii). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

The SAB finds that the calculations in the draft assessment of the PODs and OSFs for cancer endpoints are not clearly described, and the SAB expresses concerns about whether these are scientifically supported. Specifically, the SAB has concerns regarding the data used to derive the cancer POD, the rationale for restricting modeling to the multistage model, and the conditions under which the Agency's MS-COMBO multi-tumor modeling methodology provides a valid POD and cancer slope factor estimate.

The draft assessment discusses two modes of action for cancer, genotoxicity and cell proliferation, and concludes the mode of action leading to the increased incidence of liver and lung tumors is not known since the limited available experimental data do not support these hypothesized MOAs. The SAB agrees with this conclusion. However, there are publications (Watanabe et al. 2006; Young and Bordey, 2009) in the literature that propose potential connection between GABA and cell proliferation. This potential connection should be discussed in the assessment for completeness. The SAB also agrees that without a clear mode of action, the linear low-dose extrapolation method recommended in the EPA 2005 Cancer Guidelines should be used in the draft assessment.

However, the SAB has concerns with the low incidence of liver tumors (hepatocellular adenomas and carcinoma) in female mice and its impact on dose-response modeling. As indicated in

Section 1.2.5 of the draft assessment, the 1.5% incidence of liver tumors in the control B6C3F1 mice is unusually low. This was reported by the study authors as significantly lower than reported for historical controls, and at the low end of the range of the incidences in control females reported for this mouse strain by the National Toxicology Program (NTP) (mean 8%, range 0-20%). This low control incidence could significantly influence the estimate of the POD. The SAB recommends that for liver cancer, additional BMD modeling using other available models in the BMDS software package (i.e., a sensitivity analysis) be performed to examine and illustrate the impact of low concurrent controls on model choice and POD estimate.

The draft assessment discusses concerns that inclusion of the highest dose in the dose-response model for liver tumors for the B6C3F1 mouse study may impact the POD estimate and estimated cancer slope factor. The previous RDX risk assessment excluded the high dose used in this study in deriving the POD and cancer slope factor. (The SAB recognizes that the draft assessment used updated liver tumors data from female mice from PWG reanalysis, and has lower tumor incidence. Thus, the data sets used are not the same). A change in the highest dose at week 11 due to high mortality was reported, and mice that died prior to week 11 were excluded from the analysis. This results in a reduced sample size for the highest dose group from 65 to 31 animals and subsequent increased uncertainty in the response to this dose. While survival times of mice in the highest dose group were not significantly different from other dose groups, high mortality in the early weeks may mean that remaining survivors had other differences that potentially resulted in higher resistance to cancer. Excluding the highest dose group results in a fitted multistage model form that is almost linear. Using this fitted model produces an estimated POD that is much lower than that estimated with the highest dose group included. This lower POD in turn produces an unrealistically high cancer slope estimate (see Figure D-15). The SAB has no specific recommendation on how EPA should address this issue other than to include/exclude the highest dose in the sensitivity analysis for examining the effect of the highest dose on the model choice and the POD estimate.

The draft assessment relies on the multistage model to describe dose-response relationships and subsequently to estimate the POD and cancer slope factor. As discussed in the BMD guidance document (USEPA, 2012a), the IRIS program prefers to use a multistage model for cancer dose-response modeling of cancer bioassay data, when no biological basis for model form is available. The EPA considers the multistage model sufficiently flexible to address the typical dose-response patterns of cancer bioassay data, and its use encourages comparability across IRIS assessments. In its present form the draft assessment does not include a rationale for using the multistage model, and this omission leads the SAB to question the validity and scientific adequacy of other aspects of the dose-response modeling, as well as the use of the MS-COMBO package. Furthermore, the SAB concludes that more discussion on the rationale for using the multistage model and how the EPA typically assesses the multistage model fit would greatly improve clarity of presentation and reduce confusion regarding model selection. Although a discussion of the benefits and weaknesses of the multistage modeling approach is also included in the BMD guidance document, a summary of these should also be provided in the assessment. The SAB also recommends that the assessment discuss the adequacy of the fit of the multistage model to available data. This discussion could be further supported by exploring and reporting fits to other standard BMD model forms available in the BMDS software package.

The SAB expresses concerns that the *assumption of independence of tumor incidence* that is required by the MS-COMBO methodology used in estimating the POD is not clearly described. In addition, there is no discussion on the validity of this assumption for the available RDX data. However, these data are available in the pathology report in Lish et al. (1984), and concurrent incidence of liver and lung tumors was found in only one animal in the 175/100 mg/kg-day dose group and in one animal in the 35 mg/kg-day dose group, demonstrating that the assumption of independence of tumor incidence holds. Finally, the SAB cannot determine whether the MS-COMBO methodology requires that the dose-response for each tumor be adequately described by a multistage model, and whether the tumor incidence data being combined must adequately fit the same multistage model form. The SAB recommends that a better, and more detailed description of the MS-COMBO methodology be provided in the draft assessment, and that this description clarify the points raised above. In particular, text that better describes the independence assumption and the impact of violations of this assumption on the estimated POD should be included.

Key Recommendations

- For liver cancer, perform and discuss results from additional BMD modeling (i.e. a sensitivity analysis documented in the Supplemental Materials) that examines and discusses:
  - The impact of low concurrent controls on model choice and the POD estimate.
  - The effect of including/excluding the highest dose on model choice and the POD estimate.
- Provide details and discuss the adequacy of the fit of the multistage model to available data.
- Provide a better and more detailed description of the MS-COMBO methodology (in the Supplemental Information document) and ensure that this description discusses the issues below:
  - importance of the assumption of independence,
  - why this assumption is needed,
  - how this assumption might be examined statistically given adequate study data/documentation,
  - whether the independence of tumor incidence assumption further constrains the tumor-specific dose-response model form to be the same across included tumor types, and,
  - the extent to which violations of the independence of tumor incidence assumption negatively affect the estimated POD.

Suggested Recommendations

- Fit other standard BMD model forms to available data and include these findings as part of the discussion of model adequacy.
- Include a summary (in the Supplemental Information document) of the benefits and weaknesses of the multistage modeling approach.

3.4. Dose-Response Analysis

3.4.1.Oral Reference Dose for Effects other than Cancer.

Charge Question 4a. The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

EPA has done a reasonably good job describing the process and choices made to arrive at the oral RfD. The SAB concludes that the overall RfD for RDX should be based on nervous system effects. Neurotoxicity was observed in multiple animal studies and in exposed humans, and included hyperactivity, hyperirritability, tremors and convulsions. Mechanistic data supports the neurotoxic effects of RDX, namely binding to the $GABA_AR$ and antagonizing GABA-mediated post-synaptic inhibition. EPA also provides an RfD based on suppurative prostatitis and another based on testicular degeneration. The SAB finds that suppurative prostatitis, which EPA describes as a surrogate for the effects of RDX on the kidney and genitourinary system, is not an appropriate toxicological endpoint for the overall RfD. There is no known mechanistic link between suppurative prostatitis and renal papillary necrosis or adverse effects on renal function. Thus, suppurative prostatitis does not provide any indication of adverse effects in the kidneys. The SAB also concludes that testicular degeneration was not an appropriate endpoint to serve as a basis for the overall RfD. Testicular degeneration was reported at terminal sacrifice (i.e., 24 months) in one 2-year dietary study in mice (Lish et al. 1984). The SAB notes that testicular histopathology should have been seen at earlier time points (e.g., the six and 12 months' interim sacrifices) in Lish et al. (1984), as these exposure durations were several times longer than the 1.4-month duration of spermatogenesis in mice. Germ cell degeneration was also observed in a 2-year dietary study in rats (Levine et al. 1983) but only at the 12-month interim sacrifice, and not at the six-month interim or 24-month terminal sacrifice. Furthermore, testicular degeneration was not observed in most of the dietary and gavage studies in rodents (five of seven showed no effect).

While the SAB agrees that neurotoxicity should be the basis for an overall RfD for RDX, and supports the selection of the Crouse et al. (2006) study, the SAB found that the rationale for selection of the Crouse study over Cholakis et al. (1980) needs to be further clarified. The SAB also finds that the scientific support for the proposed oral RfD is somewhat lacking, as detailed in the concerns regarding the choice of BMR and the choice of some uncertainty factors.

EPA chose the Crouse et al. (2006) study over the Cholakis et al. study for several stated reasons: lack of specific monitoring for neurological effects (e.g., convulsions) in the Cholakis et al. study; a higher purity of test material in the Crouse study; fewer dose groups and wider spacing of dose groups in Cholakis et al. (1980) compared to the Crouse study; and longer exposure duration in Crouse et al. (90 d) compared to Cholakis et al. (14 d). The SAB acknowledges that the greater purity of the test material in the Crouse study is an important issue that should impact the choice of the key study. The more informative dose spacing in Crouse et al. (2006) can potentially allow for less uncertainty in dose-response modeling. The exposure duration was considerably longer in Crouse et al. (2006), and a longer exposure duration may show effects at lower doses than studies of shorter duration, even in the same dose range. While acknowledging that

the Crouse study was a better designed study to detect neurological effects, and that the monitoring for neurological effects in Cholakis et al. (1980) was incomplete, the observation of a (single) rat with convulsions at 2 mg/kg-day appeared to be a valid observation that could potentially have resulted in a lower LOAEL.

EPA raised an additional quality consideration with respect to the Cholakis et al. (1980) teratology study during the SAB review noting the observation of a single incident of convulsion in the positive control group treated with hydroxyurea. In the Cholakis et al. study, RDX elicited convulsions in pregnant rats in a dose-related manner, consistent with other toxicological studies with RDX. Hydroxyurea, a known teratogen and consequently a positive control substance, is also known to target the central nervous system (fetal and adult) (IARC, 2000). EPA cited the lack of convulsions in rats and dogs after repeated oral dosing in Morton et al. (2015), as evidence that hydroxyurea does not cause convulsions in laboratory animals. It should be noted, however, that other evidence of central nervous system stimulation; mainly aggression, was observed in this study in male rats given 1,500 mg/kg-day hydroxyurea by gavage. Additionally, group sizes in the Morton et al. study were small (n= 3 to 5 per sex) such that the power of the study to identify a rare effect e.g., convulsions, was insufficient. Hence, the Cholakis et al. study observation of convulsions in one of the positive control animals is a plausible finding, and does not negate the convulsions observed in RDX treated animals.

However, an additional study quality consideration regarding the Cholakis et al. (1980) study raised during the SAB review is the potential lack of uniformity/homogeneity of the dosing preparations (see discussion in Section 3.3.1.2). Since measures were taken by Crouse et al. to reduce the variation in dosing suspensions, it is likely that the intended dose levels were more accurately administered in the Crouse study compared with the Cholakis study, where both under-dosing and over-dosing of animals is a concern due to difficulty in maintaining uniform dose suspensions.

Given the quality issues identified for the Cholakis et al. (1980) study (with some of those issues articulated in the EPA draft assessment, and with SAB's concern described above regarding the high variability of dose levels based on the difficulty in maintaining homogeneous dosing suspensions), it is appropriate to give more weight to the Crouse et al. (2006) study with respect to the quantitative dose-response analysis. A POD derived from the Cholakis et al. study should be regarded as a low confidence value given the uncertainty regarding the actual doses administered and the wide (10x) dose spacing used in Cholakis et al. (1980), albeit recognizing that the study is of some value for RDX hazard characterization. With respect to whether pregnancy is a sensitive physiological state for the neurotoxicity of RDX, the SAB agrees that the question cannot be resolved by the available data, and notes that this uncertainty should be considered in selecting the UF_H for intraspecies variability.

Before the full scope of quality issues associated with the Cholakis et al. was identified, the SAB considered options to specifically and quantitatively take the NOAEL and LOAEL from Cholakis et al. (1980) into account.

1. Conduct a benchmark dose analysis on the convulsion data from Cholakis et al. (1980).

EPA provided information that the incidence of convulsions at the high dose in the Cholakis et al. study was combined with the incidence of other neurologic effects. The response at this dose is, therefore, not appropriate for inclusion in benchmark dose modeling. However, elimination of the high dose from the Cholakis et al. dose-response data leaves only one effective dose and this does not provide an adequate basis for dose-response modeling. Therefore, the SAB rejected this option.

2. Combine the dose-response data from Cholakis et al. (1980) and Crouse et al. (2006)
The SAB considered that it may be possible to more specifically account for the data from pregnant dams in the Cholakis et al. study by combining the data with those from Crouse et al. (2006). There were several impediments to this approach. The two studies differ in exposure duration (Cholakis et al. (1980),14-day; Crouse et al. (2006), 90-day) and sex/pregnancy status (Crouse et al. males and females; Cholakis et al. pregnant females only) providing no common factors on which to combine results. Therefore, the SAB also rejected this option.

3. Use the NOAEL (0.2 mg/kg/d) from Cholakis et al. (1980) as the POD
The SAB originally considered the advantages of using the NOAEL from Cholakis et al. as a POD to derive the oral RfD, without full consideration of potential inaccuracies in the doses administered in the study. As noted above, with the elimination of the high dose from Cholakis et al. (due to inclusion of non-convulsive effects), there is no basis for benchmark dose modeling, and a NOAEL is an appropriate basis for a POD. The use of the NOAEL from Cholakis et al. as the basis for the RfD eliminates issues concerning the choice of a BMR from Crouse et al. (2006) (see response to charge question 3a(iii) in Section 3.3.1.3), and addresses the SAB's concern with the existence of a lower LOAEL from Cholakis et al. However, given the quality issues associated with the Cholakis et al. study, the SAB places more weight on the Crouse et al. (2006) study for the derivation of a POD, and therefore also rejected this option to use the NOAEL of the Cholakis study.

4. Use the Dose-Response Data from Crouse et al. (2006) as the primary basis of deriving the RfD
The SAB recommends using the data from Crouse et al. (2006) in a benchmark dose analysis to derive a POD for the RfD. While as noted above, an RfD derived from the NOAEL of Cholakis et al. (1980) is not recommended, it is shown for comparison to the RfD derived from Crouse et al.

Table 4 below provides a comparison between EPA's proposed value (first row entry) based on the 1% BMR from Crouse et al. (2006) with alternate RfDs. This is meant to provide several possible pathways for EPA to consider in revising the RfD. If the same UFs (composite UF of 100) are applied to the PBPK-adjusted NOAEL POD from the Cholakis et al. study as EPA applied to the PBPK-adjusted BMDL POD from the Crouse et al. study, the RfD based on Cholakis et al. (1980) would be 1×10^{-3} mg/kg/day. Applying the SAB recommended composite UF of 300 to the PBPK-adjusted NOAEL POD from Cholakis et al. (1980) results in an RfD of 3×10^{-4} mg/kg-day. Applying the SAB-recommended composite UF of 300 to the 1% BMR from Crouse et al. would result in an RfD of 1×10^{-3} mg/kg-day. If, however, the RfD from Crouse et al. were calculated based on a BMR of 5% as discussed by the SAB (see response to charge question 3a(iii) in Section 3.3.1.3), applying the recommended composite UF of 300, the RfD

would be 4 x 10^{-3} mg/kg-day. The SAB had considered the value of an UF that could be used to address the frank neurological effect, which was the reason EPA chose a 1% BMR in their draft assessment, if EPA were to use a BMR of 5% rather than 1%. However, there are other considerable uncertainties in the database, including the lack of testing for developmental neurotoxicity and proximity of convulsive doses to lethal doses. Therefore, the SAB concludes that the full default UF_D of 10 should be used with a BMR of 1% or 5%, and the use of this uncertainty factor should be sufficient to account for the uncertainty caused by the use of a 5% BMR for a frank effect. These RfDs can be compared to the EPA's proposed RfD of 3 x 10^{-3} mg/kg-day from Crouse et al. based on a BMR of 1% and a composite UF of 100.

Table 4. Comparison of Derived Candidate RfD values using different PODs and composite uncertainty factors.

Reference	POD (mg/kg-day)	POD Type	POD_{HED}[a]	Composite UF	RfD value (mg/kg-day)
Crouse et al (2006)	0.57	$BMDL_{01}$	0.28	100	0.003[c]
Cholakis et al. (1980)	0.2	NOAEL	0.097	300	0.0003[d]
Crouse et al. (2006)	0.569	$BMDL_{01}$	0.28	300	0.001
Crouse et al. (2006)	2.66[b]	$BMDL_{05}$	1.295	300	0.004

a POD_{HED} is calculated from POD x PBPK derived adjustment factor of 0.487
b $BMDL_{05}$ estimate is from Table 2 in Section 3.3.1.3
c EPA proposed RfD
d Not recommended by the SAB, but included here for comparison purpose

Consideration of mortality

The SAB interprets this charge question as asking whether an RfD based on convulsions (from either Crouse et al. (2006), or Cholakis et al. (1980)) is adequately protective against lethality. The SAB agrees that mortality and convulsions are linked. However, the SAB is not aware of any evidence for RDX or similar seizurogenic compounds where neurologic mortality occurs in the absence of convulsions. The overall candidate RfDs (Table 4) can be compared to the NOAEL for convulsions of 10 mg/kg-day with no mortality in the monkey study of Martin and Hart (1974). The SAB finds that this comparison provides some confidence that an RfD based on a BMDL derived from Crouse et al. provides a margin of safety with respect to neurologic-based lethality. However, the SAB acknowledges that the Martin and Hart study had a small sample size. The SAB, therefore, strongly endorses increasing the UF_D and apply a UF_D of 10 to provide an appropriate margin of safety between convulsive and lethal neurologic effects (as well as accounting for data gaps in developmental neurotoxicity and lack of incidence data for less severe neurotoxic effects).

Key Recommendations

- The SAB agrees that the overall RfD should be based on neurotoxicity as measured by convulsions in Crouse et al. (2006), but the SAB concludes that the scientific support for the proposed oral RfD is somewhat lacking primarily due to concerns with the choice of BMR and the value of the database uncertainty factor and the uncertainty factor for subchronic to chronic extrapolation. This deficiency needs to be rectified.
- Since the Cholakis et al. (1980) study suffers from several quality issues, it is appropriate to give more weight to the Crouse et al. (2006) study with respect to the quantitative dose-response analysis. The rationale for the selection of Crouse et al. (2006) and setting aside the Cholakis et al. (1980) study even though it reported a lower NOAEL/LOAEL, should be strengthened and clarified.
- The discussion and key recommendations from Section 3.3.1.3 and Section 3.3.1.4 are all pertinent to the SAB finding that the scientific support for, and discussion of, the proposed oral RfD for the convulsions endpoint is lacking. These recommendations are repeated here:
    - EPA should consider using a BMR of 5% for their dose-response modeling of the Crouse et a. (2006) data while addressing the uncertainty of using data on a frank effect (convulsions in this case) as the basis of an RfD with a larger database uncertainty factor.
    - If EPA decides to use a BMR of 1% for the dose-response assessment using Crouse et al. (2006), EPA should justify why the greater conservatism in risk assessment required for a frank effect (due to the lack of incidence data for less severe endpoints) is better dealt with through a lower BMR than through application of UF_D.
    - If EPA decides to use a BMR of 1% for the Crouse et a. (2006), EPA should provide in its discussion clear justification for why a 1% BMR is more appropriate than a 5% BMR for RDX, given the greater uncertainty introduced into the dose-response assessment for RDX using a BMR of 1%.
    - Consistent with EPA guidance for uncertainty factors, the SAB strongly recommends that EPA apply the full default UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe neurological effects resulting in use of a severe effect (convulsions) as a basis for the RfD, and the proximity of lethal doses to convulsive doses.
    - EPA should discuss whether potential neurodevelopmental effects of RDX would be sufficiently addressed by a UF_D of 10, given that the mechanism of RDX argues there would likely be developmental neurotoxic effects and the other database uncertainties (lethality at convulsive doses, other less severe neurotoxic effects that may have a lower LOAEL) that also need to be addressed by the UF_D.
    - EPA should reconsider the value of the UF_S and at a minimum provide stronger justification for a UF_S of 1.

3.4.2.Inhalation Reference Concentration for Effects other than Cancer

Charge Question 4b. The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

There are no toxicokinetic data from inhalation exposure of laboratory animals or humans to RDX. There are epidemiological studies of persons exposed occupationally to RDX (Ma and Li, 1993; Hathaway and Buck, 1977), but no information provided on exposure levels. These workers were likely exposed dermally and by inhalation.

Key Recommendation

- EPA should not attempt to derive an inhalation reference concentration since neither toxicokinetic data nor exposure levels information from animal or human RDX inhalation studies are available to make estimation possible.

3.4.3. Oral Slope Factor for Cancer

Charge question 4c. The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

The SAB finds that the calculation of an OSF for cancer endpoints in the draft assessment is not clearly described and, in keeping with the discussion in Section 3.3.5.3, the SAB expresses several concerns regarding whether the method used to derive the OSF is scientifically supported. The SAB makes multiple suggestions for how the discussion on the derivation of the OSF can be improved.

The OSF is estimated as the plausible upper-bound (95% upper CI) for the true slope, or risk per unit dose, from which the probability that an individual will develop cancer if exposed to an agent for a lifetime of 70 years can be derived. In practice, and as presented in the draft assessment, the OSF for the cancer endpoint is obtained as the slope of the line from a POD, in this case the BMDL for 10% BMR ($BMDL_{10}$), to the estimated control response at a dose of zero. Consequently, any changes to the derivation of the POD will be reflected in the estimate of the OSF. In its response to question 3e(iii), the SAB identifies issues with the data used to derive the cancer POD, and offers recommendations for improving the calculation of the POD (see Section 3.3.5.3). These recommendations (e.g. with vs. without the highest dose group in dose-response modeling) will change the estimated POD and thus the OSF.

The draft assessment proposes combining tumors from different sites in determining an overall cancer risk. The SAB finds that this is both logically and toxicologically sound. While not discussed in either the draft assessment or the supplemental material, the independence of tumor location is a key assumption for scientifically appropriate use of the MS-COMBO model. The SAB considered the original study data provided in the draft assessment, and agrees with the EPA that there is no biological or statistical support for the notion that the two tumors used in the MS-COMBO analysis are interdependent. Hence the EPA's assumption of independence of the two tumor sites and the MS-COMBO approach are considered valid. However, as discussed in Section 3.3.5.1, a significant increase in lung tumors in female mice was only seen at the highest dose, which exceeded the MTD. Furthermore, as discussed in Section 3.3.5.3, additional issues around the use of the MS-COMBO model remain to be clarified.

The SAB expresses concern that the near linearity of the fitted multistage dose-response models (see Table 2-7 in the draft assessment identifying all selected models as Multistage 1°) results in a relatively poor fit (model estimates) at the highest doses. The two fitted models used (see Figures D-12 and D-14 in the RDX draft supplement document) have $BMDL_{10}$ estimates that are larger than the two lowest non-zero doses used. The Cancer Guidelines (USEPA, 2005) states (page 3-16): "*If the POD is above some data points, it can fail to reflect the shape of the dose-response curve at the lowest doses and can introduce bias into subsequent extrapolations*." This seems to be what is happening with the data on RDX-induced adenomas and carcinomas, and the issues with the $BMDL_{10}$ seem to arise primarily because the fitted multistage models (with parameter constraints invoked) lack sufficient curvature. Larger than expected $BMDL_{10}$ values (the PODs) result in lower estimated OSFs. The SAB conjectures that using a model form that allows more curvature could provide a better fit at the mid-range and higher doses, and improve the quality of fit. As mentioned in Section 3.3.5.3, the SAB acknowledges that EPA's standard practice is to use the multistage model for benchmark dose modeling of cancer dose-response when there is no biological basis for choosing another model. In this case however, the relatively poor fit of the multistage model to the hepatocellular and alveolar/bronchiolar adenomas and carcinomas data produces an estimate of the POD with poor properties. The SAB recommends that at a minimum, the assessment discuss the adequacy of the fit of the multistage model to available data**.** This discussion could be further supported by exploring and reporting fits to other standard BMD model forms – engaging in a curve-fitting exercise starting for example with the list in Table D-13 in the draft supplemental document. Although the multistage model does ensure positive slopes throughout, the BMDS software facilitates fitting other models that also adhere to this constraint.

The SAB expresses concern that the results for liver cancer in concurrent female control mice were very low (1.5 %) compared to available historical control incidence (8%; range 0-20%) (page 1-62 of the draft assessment). This low rate influences the final model for liver tumor incidence, which in turn significantly impacts the estimate of the POD and hence the OSF. It is not clear how this issue impacts the POD estimate derived via the MS-COMBO analysis where liver tumor results are combined with those of lung tumors to produce the final POD used. The SAB recommends that EPA acknowledge the low concurrent control liver tumor incidence in female mice and discuss its impact on the level of confidence in the final MS-COMBO estimate of the proposed POD.

The SAB also notes, and the draft assessment confirms (Section 1.2.5, page 1-61), that at the highest dose level in the Lish et al. (1984) study for the first 11 weeks, the animals had an elevated mortality strongly suggesting that the maximum tolerated dose had been greatly exceeded. At 11 weeks, the researchers lowered the dose, and it was a duration-weighted average dose level that was used as the highest dose in the fitting of the multistage model (see Section D.2.2 (pages D-31 to D-33) of the RDX draft supplement document). The Cancer Guidelines (page A-4) discuss this situation and offer that the decision to use or not use data from doses that exceed the MTD is "*a matter of expert judgement*". The SAB has concerns that including this dose significantly impacts the final estimated POD. The SAB recommends that additional insight be sought by fitting the multistage model and estimating the POD after exclusion of this dose level, comparing the POD generated from both models, and discussing why the estimate that is based on the use of the highest dose data is preferred. Following this analysis through to the MS-COMBO

results seems scientifically appropriate. This comparison and subsequent discussion is supported by the fact that the current IRIS entry for RDX of 0.11 per mg/kg-day was determined using the liver tumor data (Lish et al. 1984) with the highest dose values excluded.

Key Recommendation

- Acknowledge that the issues with the estimation of the POD for estimation of the cancer slope factor as discussed in Section 3.3.5.3 and note that the associated key recommendations for improving the presentation on the POD also apply to the estimation of the cancer slope factor.

3.4.4. Inhalation Unit Risk for Cancer

Charge Question 4d. The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

There are no toxicokinetic data from inhalation studies of RDX in laboratory animals or humans, no inhalation carcinogenicity bioassays of RDX, nor data on cancer incidence in humans. Therefore, an inhalation unit risk for cancer cannot be derived.

Key Recommendations

- EPA should not attempt to derive an inhalation unit risk since there are no study data available to make estimation possible.

3.5. Executive Summary

Charge Question 5. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Generally, the SAB considered the Executive Summary to be well-written, succinct, and clear. As changes are made to the body of the draft assessment in response to the SAB's recommendations, the Executive Summary should be updated accordingly. In addition, the SAB provides the following suggested recommendations for improving the Executive Summary.

Suggested Recommendations

- On the characterization and description of urogenital system hazard and risk.
  - Do not use the suppurative prostatitis as a surrogate for kidney and other urogenital system effects in males. Other urogenital system effects are of more importance and should be described. The description of the urogenital effects in male rats should include specific mention of the renal effects (i.e. renal papillary necrosis and associated renal inflammation), not the prostatic effects.
  - An RfD based on suppurative prostatitis should be derived as a stand-alone endpoint, and a separate RfD should be derived for kidney and other urogenital system effects. Because the observed suppurative prostatitis is part of a larger spectrum of prostatic inflammatory changes that are frequently found in aged F344 rats, the dose-response for this le-

sion found in male rats may in fact not reflect the overall incidence of all types of prostatitis combined in each dose group. The prostatic inflammation and renal/bladder effects may be inter-related, but this only occurs at the highest RDX dose tested, and there seems to be no basis for the assertion that suppurative prostatitis is a "surrogate marker" for renal/bladder effects. This change does not affect the overall oral reference dose because that is based on the nervous system effects.

- In the section on "Suppurative prostatitis," the possibility of a bacterial infection is raised and its potential significance to RDX toxicity is briefly discussed. However, it should also be noted that this inflammatory effect could also occur without a bacterial infection.
- P xxiii line 7 – 9: The first sentence indicates human potential for kidney and urogenital toxicity, which is justified, but indicates this is "based on" increased relative kidney weights and histopathological changes. P 1-24 lines 24-30 indicates inconsistent findings in the subchronic studies and down-plays the organ weight findings in the chronic studies, so the executive summary is inconsistent with this.

- Regarding the description of animal cancer bioassay results, the following should be added to indicate some of the uncertainty or limitations in the animal cancer bioassay results.
  - In the Summary, add "limited" to the sentence -. "*Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on limited evidence of positive trends in liver and lung tumor incidence in experimental animals."*
  - In the body, add clarifying or cautionary language on page xxv, line 26 such as *Despite limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk....* or *Cognizant of limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk....*

- On other content clarification, the following missing information should be included and the following editorial comments should be addressed:
  - P xxvii line 23 – 25: Please clarify the meaning of "more representative of potential human exposures," and be explicit regarding the uncertainty associated with identifying a representative experimental exposure. It is not clear, given the limited information on RDX exposures in the Preface or elsewhere in the draft assessment, that dietary exposure is "*more representative of potential human exposures*". It seems possible that human exposures could involve different or varied sources of RDX exposure (e.g., on swallowed dust particles, consumed soil, incorporated into plants) such that neither experimental exposures as diet nor as gavage would be obviously "representative" and both experimental approaches to exposure (dosing) would include uncertainty.
  - Explain the importance of *RDX purity* in published studies.
  - List the main criteria used in choosing the principal study.
  - Include a discussion of the concordance in doses producing convulsions and doses at which death occurred in key animal studies in the brief discussion of neurologic effects in the section entitled "Effects other than cancer observed during oral exposure." The lethality associated with convulsions is currently not mentioned.
  - Provide a summary statement addressing the confidence (i.e., low, medium or high) in the RfD.

- On page xxiii, a paragraph break is needed after the sentence, "*There is no known MOA for male reproductive effects of RDX exposure.*" The next sentence does not relate to the male reproductive effects but speaks to the evidence for effects in other organs/systems.
- Combine the paragraphs found on page xxv entitled "Effects other than cancer observed following inhalation exposure" and "Inhalation reference concentration (RfC) for effects other than cancer." There is no available literature to support the identification of hazards following inhalation and a reference concentration cannot be determined. This can be stated simply in a single paragraph.

4. FUTURE RESEARCH NEEDS

4.1. Metabolism

As discussed in Section 3.1, the metabolism of RDX has not been adequately studied. Toxicity information on metabolites such as MNX, TNX, MEDINA, and NDAB are limited or non-existent. More research on the metabolism of RDX to identify metabolites and their potential toxicity is needed to improve the risk assessment of RDX.

4.2. Intrahuman Variation

Data on inter-subject variability in receptor binding and response are needed to move away from the current default UF_H of 10.

4.3. Nervous System Effects

As discussed in Section 3.3.1, sufficiently sensitive test batteries to detect neurobehavioral consequences produced by chronic/subchronic exposure to RDX, especially during pregnancy, have not been conducted. Moreover, tests designed to detect subtle developmental neurotoxicity during the perinatal-weaning period have also not been conducted. These significant data gaps need to be addressed as follows:

- The SAB strongly recommends that developmental neurotoxicity studies be conducted in animals. These studies should include test batteries to detect potential fine psychomotor impairments, anxiety and social impairments, decreased executive functioning and long-term memory.
- Data needs for improving the risk assessment of RDX include behavioral and morphometric studies that can permit more accurate assessments of RDX exposures to the developing nervous system at subconvulsive dose.
- Studies with adequate power to address cognitive and behavioral effects, as well as developmental neurotoxicity of RDX, and to establish relevant dose-response relationships.

Additonal data gaps that need to be addressed include:

- Additional dose specifications (levels) should be examined to provide a more reliable dose-response relationship for convulsions and other neurotoxic effects of RDX.
- Studies to determine whether pregnant rats are more sensitive to RDX than non-pregnant rats.
- More definitive study to look at effects of exposure duration that can better inform subchronic to chronic extrapolation.

REFERENCES

Angerhofer, R; Davis, G; Balezewski, L. (1986). Teratological assessment of Trinitro-RDX in rats. (75-5100573-86). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency.

Arfsten, DP; Davenport, R; Schaeffer, DJ. (1994). Reversion of bioluminescent bacteria (Mutatox) to their luminescent state upon exposure to organic compounds, munitions, and metal salts. *Biomed Environ Sci* **7,** 144-149.

Bach, PH; Nguyen, TK. (1998) Renal papillary necrosis--40 years on. *Toxicol Pathol*. 26(1):73-91.

Bannon, DI; Dillman, JF; Phillips, CS; Perkins, EL. (2009). Global gene expression in rat brain and liver after oral exposure to the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Chem Res Toxicol* 22: 620-625.

Beyer, BK; Chernoff, N; Danielsson, BR; Davis-Bruno, K; Harrouk, W; Hood, RD; Janer, G; Wandel LU; Kim, JH; Rocc,a M; Rogers J; Scialli, AR. (2011) ILSI/HESI Maternal Toxicity Workshop Summary: Maternal Toxicity and Its Impact on Study Design and Data Interpretation. *Birth Defects Research* (Part B). 92:36–51.

Beller, HR; Tiemeier, K. (2002). Use of liquid chromatography/tandem mass spectrometry to detect distinctive indicators of in situ RDX transformation in contaminated groundwater. *Environmental Science & Technology* 36: 2060-2066.

Bhushan, B; Trott, S; Spain, JC; Halasz, A; Paquet, L; Hawari, I. (2003). Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a rabbit liver cytochrome P450: Insight into the mechanism of RDX biodegradation by Rhodococcus sp. Strain DN22. *Appl Environ Microbio* 69: 1347-1351. http://dx.doi.org/10.112B/AEM.69.3.1347-1351.2003

Brix, AE. (2002) Renal papillary necrosis. *Toxicol Pathol*.30:672-674.

Bronson, FH; Desjardins, C. (1977). Reproductive failure in aged CBF_1 male mice: Interrelationships between pituitary gonadotropic hormones, testicular function, and mating success. *Endocrinology* 101: 939-945.

Bruni, JF; Huang, HH; Marshall, S; Meites, J. (1977). Effects of single and multiple injections of synthetic GnRH on Serum LH, FSH and testosterone in young and old male rats. *Biol Reprod* 17: 309-312.

Bruyns-Haylett, M; Luo, J; Kennerley, A; Harris, S; Boorman, L; Milne, E; Vautrelle, N; Hayashi, Y; Whalley, BJ; Jones, M; Berwick, J; Riera, J; Zheng, Y. (2017). The neurogenesis of P1 and N1: A concurrent EEG/LFP study. *NeuroImage* 146: 575-588.

Cao, CI; Reddy, G; Bannon, DI; Hohnson, MS. (2008). In vitro study of hexahydro-1,3,-5-trinitro-1,3,5-triazine (RDX) metabolism in human liver. Aberdeen Proving Ground, ND: U.S. Army Center for Health Promotion and Preventive Medicine.

Carney, EW; Kimmel, CA, (2007) Interpretation of Skeletal Variations for Human Risk Assessment: Delayed Ossification and Wavy Ribs. *Birth Defects Research (Part B*)- *Developmental and Reproductive Toxicology* 80:473-496.

Chen, H; Hardy, MP; Zirkin BR. (2002). Age-related decreases in Leydig cell testosterone production are not restored by exposure to LH in vitro. *Endocrinology* 143: 1637–1642.

Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-8027). Kansas City, MO: Midwest Research Institute.

Cohen, BJ; Anver, MR; Ringler, DH; Adelman, RC. (1978). Age-associated pathological changes in male rats. *Federation Proc* 37: 2848-2850.

Coleman, NV, Spain, JC, Duxbury, T. (2002). Evidence that RDX biodegradation by Rhodococcus strain DN22 is plasmid-borne and involves a cytochrome p-450. *J Appl Microbiol* 93: 463-472.

Costa, LG; Giordano, G; Faustman, EM. (2010). Domoic acid as a developmental neurotoxin. *Neurotoxicology* 33: 409-423.

Creasy, D; Bube , A; de Rij, KE; Kandori, H; Kuwahara, M; Masson, R; Nolte, T; Reams, R; Regan, K; Rehm, S; Rogerson, P; Whitney, K. (2012). Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol Pathol*. 40(6 Suppl):40S-121S.

Creeley, CE. (2016). From drug-induced developmental neural apoptosis to pediatric anesthetic neurotoxicity – where are we now? *Brain Sciences* 6:32-44

Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Subchronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Dey, A; Kang, X; Qiu, J; Du, Y; Jiang, J (2016). Anti-Inflammatory Small Molecules To Treat Seizures and Epilepsy: From ench to Bedside. *Trends in Pharmacological Sciences* 37: 463-484.

Doucette, TA; Tasker, R. (2016) Perinatal domoic acid as a neuroteratogen. *Curr Top Behav Neurosci*. 29:87-110.

Eyo, U. B; Murugan, M; Wu, LJ. (2017), Microglia–Neuron communication in epilepsy. *Glia* 65: 5–18. doi:10.1002/glia.23006

Fellows, RJ; Driver, CR; Cataldo, DA; Harvey, SD. (2006). Bioavailability of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the prairie vole (Microtus ochrogaster). *Environmental Toxicology and Chemistry* 25:1881-1886.

Fernandez, S; Loddenkemper, T; Galanopoulou, AS; Moshe, SL (2015). Should epileptiform discharges be treated? *Epilepsia* 56: 1492-1504.

Fuller, ME; Perreault, N; Hawari, J. (2010). Microaerophilic degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by three Rhodococcus strains. *Letters in Applied Microbiology* 51:313–318.

Fuller, ME; McClay, K; Hawari, J; Paquet, L; Malone, TE; Fox, BG; Steffan, RJ. (2009). Transformation of RDX and other energetic compounds by xenobiotic reductases XenA and XenB. *Appl Microbiol Biotechnol* 84:535-544.

Galineau, L; Kas, A; Worbe, Y; Chaigneau, M; Herard, AS; Guillermier, M; Delzescaux, T; Féger J, Hantraye, P; Tremblay, L. (2017). Cortical areas involved in behavioral expression of external pallidum dysfunctions: A PET imaging study in non-human primates. *Neuroimage*.146:1025-1037.

Garcia-Reyero, N; Habib, T; Pirooznia, M; Gust, KA; Gong P; Warner, C; Wilbanks, M; Perkins, E. (2011). Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. *Ecotoxicology* 20:580-594.

George, SE; Huggins-Clark, G; Brooks, LR. (2001). Use of a Salmonella microsuspension bioassay to detect the mutagenicity of munitions compounds at low concentrations. *Mutat Res* 490: 45-56.

Gill, DA; Bastlund, JF; Watson, WP; Ryan, CL; Reynolds, DS; Tasker, RA. (2010). Neonatal exposure to low-dose domoic acid lowers seizure threshold in adult rats. *Neuroscience* 169: 1789-1799.

Gosden, RG; Richardson, DW; Brown, N; Davidson, DW (1982). Structure and gametogenic potential of seminiferous tubules in ageing mice. *J Reprod Fert* 64: 127-130.

Grant, KS; Burbacher, TM; Faustman, EM; Gratttan, L. (2010). Domoic acid: neurobehavioral consequences of exposure to a prevalent marine biotoxin. *Neurotoxicol Teratol.* 32:132-141.

Grasso, C; Li Volsi, G; Cataldo, E; Manzoni, D; Barresi, M. (2016). Effects of bicuculline application on the somatosensory responses of secondary vestibular neurons. *Neuroscience* 335:122-133.

Guo, L; Xu, H; Chen, Y; Chang, Y. (1985). Distribution and metabolism of tritium-labeled hexogen in white mice, *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 3: 335.330.

Gust, KA; Brasfield, SM; Stanley, JK; Wilbanks, MS; Chappell, P; Perkins, EJ; Lotufo, GR; Lance, RF. (2011). Genomic investigation of year-long and multigenerational exposures of fathead minnow to the munitiont compound RDX. *Environmental Toxicology and Chemistry* 30:1852-1864.

Halasz, A; Manno, D; Perreault, NN; Sabbadin, F; Bruce, NC; Hawari, J. (2012). Biodegradation of RDX Nitroso Products MNX and TNX by Cytochrome P450 XplA. *Environmental Science and Technology* 46:7245-7251.

Hart, ER. (1976). Two-year chronic toxicity study in rats. (N0014-73-C-0162). Kensington, MD: Litton Bionetics, Inc.

Haseman, JK; Huff, JE; Rao, GN; Arnold, JE; Boorman, GA; McConnell, EE. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice. *J Natl Cancer Inst* 75: 975-984.

Hathway, JA; Buck, CR. (1977). Absence of health hazards associated with RDX manufacture and use. *J Occup Med* 19: 269-272.

Hess-Ruth, A; Crouse, L; Roszell, L. (2007). RDX pilot development neurotoxicity test in rats. (Toxicology Study No. 85-XC-064Y-07). Aberdeen Proving Grounds: U.S. Army Center for Health Promotion and Preventive Medicine.

Hiolski, EM; Ito, S; Beggs, JM; Lefebvre, KA; Litke, AM; Smith, DR. (2016). Domoic acid disrupts the activity and connectivity of neuronal networks in organotypic brain slice cultures. *Neurotoxicology* 56:215-224.

Hollander, AI; Colbach, EM. (1969). Composition C-4 induced seizures: A report of five cases. *Mil Med* 134: 1529-1530.

Hu, Z; Li, Z (2017). miRNAs in synapse development and synaptic plasticity. *Curr Opin Neurobiol* 45: 24-31.

IARC (International Agency for Research on Cancer). (2000). Some antiviral and antineoplastic drugs, and other pharmaceutical agents. .*IARC Monographs on the evaluation of carcinogenic risks to humans* Vol 76. Pg 347 - 386. https://monographs.iarc.fr/ENG/Monographs/vol76/mono76-14.pdf;

Jaligama, S; Kale, VM; Wilbanks, MS; Perkins, EJ; Meyer, SA. (2013). Delayed myelosuppression with acute exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and environmental degradation product hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) in rats. *Toxicology and Applied Pharmacology* 266:443-451.

Jeilani, YA; Duncan, KA; Newallo, DS; Thompson, AN Jr.; Bose, NK. (2015). Tandem mass spectrometry and density functional theory of RDX fragmentation pathways: Role of ion-molecule complexes in loss of NO3 and lack of molecular ion peak. *Rapid Communications in Mass Spectrometry* 29:802-810.

Johnson, L; Neaves, WB. (1983). Enhanced daily sperm production in the remaining testis of aged rats following hemicastration. *J Androl* 4:162-166.

Kim, JY; Liu, CY; Zhang, F; Duan, X; Wen, Z; Song, J; Feighery, E; Lu, B; Rujescu, D; St Clair D; Christian, K; Callicot, JH; Weinberger, DR; Song, H; Ming, Gl. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148:1051-1064.

Krishnan, K; Crouse, LCB; Bazar, MA; Major, MA; Reddy, G. (2009). Physiologically based pharmacokinetic modeling of cyclotrimethylenetrinitramine in male rats. *J Appl Toxicol* 29: 629-637. http://dx.doi.org/10.1002/jat.1455

Kucukardali, Y; Acar, HV; Ozkan, S; Nalbant, S; Yazgan, Y; Atasoyu, EM; Keskin, O; Naz, A; Akyatan, N; Gokben, M; Danaci, M. (2003). Accidental oral poisoning caused by RDX (cyclonite): A report of 5 cases. *J Intensive Care Med* 1B: 42-46. http://dx.doi.org/10.1177/0885066602239123

Lee, E; Lee, J; Kim, E. (2016) Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders. *Biol Psychiatry* 81: 838-847.

Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM. (1981a). Thirteen week toxicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Fischer 344 rats. *Toxicol Lett* 8: 241-245.

Levine, BS; Furedi, EM; Gordon, DE; Burns, JM; Lish, PM. (1981b). Thirteen *week oral (diet) toxicity study of trinitrotoluene (TNT) and RDX in F344 rats. Fundam Appl Toxicol* 15: 3730380. http://dx.doi.org/10.1016/0272-0590(90)90062-0

Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM. (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report—phase V, Chicago, IL: IIT Research Institute.

Levine, BS; Furedi, EM; Gordon, DE; Barkley, JJ; Lish, PM. (1990). Toxic interactions of the munitions compounds TNT and RDX in F344 rats. *Fundam Appl Toxicol* 15: 373-380.

Lish, PM; Levine, BS; Fured, EM; Sagartz, JM; Rac, VS. (1984) Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes 1 and 3). U.S. Army Medical Research and Development Command, Chicago, IL.

Ma, B; Li,H. (1993). Neurobehavior effects of hexogen on exposed workers. *Gongye Weisheng yu Zhiyebin* 19**:** 20-23.

Major, MA; Reddy, G; Berge, MA; Patzer, SS; Li, AC; Gohdes, M. (2007). Metabolites profiling of [14C] hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. *J Toxicol Environ Health A* 70: 1191-1202. http://dx.doi.org/10.1080/15287390701252717

Marriott, AL; Tasker, RA; Ryan, CL; Doucette, TA. (2016). Alterations to prepulse inhibition magnitude and latency in adult rats following neonatal treatment with domoic acid and social isolation rearing. *Behav Brain Res* 298(Pt B):310-317.

Martin, D; Hart, E. (1974). Subacute toxicity of RDX and TNT in monkeys (pp. 1-216). (ADA044650). Kensington, MD: Litton Bionetics, Inc.

Marty, S; Wehrle, R; Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *The Journal of Neuroscience* 20:8087-8095.

McCormick, NG; Cornell, JH; Kaplan, AM. (1981). Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Appl Environ Microbiol* 42**:** 817-823.

Melnick, RL; Thaye, KA; Buche, JR. (2008). Conflicting views on chemical carcinogenesis arising from the design and evaluation of rodent carcinogenicity studies. *Environ Health Perspect* 116**:** 130-135.

Merrill, SL. (1968). Ingestion of an explosive material, compostion C-4: A report of two cases. *USARV Med Bull* 40: 5-11.

Meunier, CN; Chameau, P; Fossier, PM. (2017). Modulation of Synaptic Plasticity in the Cortex Needs to Understand All the Players. *Front Synaptic Neurosci* 9:2-15. doi: 10.3389/fnsyn.2017.00002.

Meyer, SA; Marchand AJ; Hight JL; Roberts GH; Escalon LB; Inouye LS; MacMillan DK. (2005). Up-and-down procedure (UDP) determinations of acute oral toxicity of nitroso degradation products of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Journal of Applied Toxicology* 25:427-434.

Mills, BD; Pearce, HL; Khan, O; Jarrett, BR; Fair, DA; Lahvis, GP. (2016). Prenatal domoic acid exposure disrupts mouse pro-social behavior and functional connectivity MRI. *Behav Brain Res* 308:14-23.

Morton, D; Reed, L; Huang, W; Marcek, JM; Austin-LaFrance, R; Norithcott, CA; Schelling, SH; Enerson, BE; Tomlinson, L. (2015). Toxicity of hydroxyurea in rats and dogs. *Toxicologic Pathology* 45: 498-512.

Mukhi, S; Patino, R. (2008). Effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in zebrafish: General and reproductive toxicity. *Chemosphere* 72:726-732.

Musick, TI; Berge, MA; Patzer, SS; Tilch, KR. (2010). Absorption, distribution, metabolism, and excretion of 14C-RDX following oral administration to minipigs (DAAD05-02-P-

2319). (ADA526472). Madison, WI: Covance Laboratories Inc. http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA526472

Nasrallah, FA; Kaur, K; Yeow, LY; Chuang, KH. (2017). GABAergic effect on resting-state functional connectivity: Dynamics under pharmacological antagonism. *NeuroImage* 149: 53-62.

Nasehi, M; Roghani, F; Ebrahimi-Ghiri, M; Zarrindast, MR. (2017). Role of the amygdala GABA-A receptors in ACPA-induced deficits during conditioned fear learning. *Brain Res Bull* 131:85-92.

Neal, GE; Judah, DJ; Hard, GG; Ito, N. (2003). Differences in ethoxyquin nephrotoxicity between male and female F344 rats. *Food Chem Toxicol* 41:193-200.

OECD (Organization for Economic Cooperation and Development) (2015). OECD guideline for testing of chemicals: Reproduction/Developmental Toxicity Screening Test (Test Guideline 421).

Pan, X; M.J. San Francisco, MI; Lee, C; Ochoa, KM; Xu, X; Liu, J; Zhang, B; Cox, SB; Cobb, GP. (2007a) Examination of the mutagenicity of RDX and its N-nitroso metabolites using the Salmonella reverse mutation assay. *Mutat Res* 629**,** 64-69.

Pan, X; Zhang, B; Smith, JN; San Francisco, M; Anderson, TA; Cobb, GP. (2007b). N-Nitroso compounds produced in deer mouse (Peromyscus maniculatus) GI tracts following hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure. *Chemosphere* 67: 1164-1170. http://dx.doi.org/10.1016/j.chemosphere.2006.10.077

Pan X; Ochoa KM; San Francisco MJ; Cox SB; Dixon K; Anderson TA; Cobb GP. (2013). Absorption, distribution, and biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine in B6C3F1 mice (Mus musculus). *Environmental Toxicology and Chemistry* 32:1295-1303.

Parker, GA; Reddy, G; Major, MA. (2006). Reevaluation of a twenty-four-month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse. *Int J Toxicol* 25**:** 373-378.

Reddy, G; Erexson, GL; Cifone, MA; Major, MA; Leach, GL. (2005) Genotoxicity assessment of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Int J Toxicol* 24**:** 427-434.

Rivera, C; Voipio, J; Payne, JA; Ruusuvuori, E; Lahtinen, H; Lamsa, K; Pirvola, U; Saarma, M; Kaila, K. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397:251-255.

Rogers, JM; Chernoff, N; Keen, CL; Daston, GP. (2005). Evaluation and interpretation of maternal toxicity in Segment II studies: Issues, some answers, and data needs. *Toxicology and Applied Pharmacology* 207: S367–74.

Salari, AA; Amani, M. (2017) Neonatal blockade of GABA-A receptors alters behavioral and physiological phenotypes in adult mice. *Int J Dev Neurosci* 57:62-71.

Schneider, NR; Bradley, SL; Andersen, ME. (1978). Distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in rat after sub-chronic administration. *Toxicol Appl Pharmacology* 46: 163-171.

Smith, JN; Pan, XP; Gentles; A; Smith, EE; Cox, SB; Cobb, GE. (2006). Reproductive effects of hexahydro-1,3,5-trinitroso-1,3,5-triazine in deer mice (Peromyscus maniculatus) during a controlled exposure study. *Environ Toxicol Chem* 25: 446-451.

Smith, JN; Espino, MA; Liu, J; Romero, NA;; Cox, SB; Cobb, GP. (2009). Multigenerational effects in deer mice (Peromyscus maniculatus) exposed to hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). *Chemosphere* 75: 910-914.

Snodgrass, HL, Jr. (1984). Preliminary assessment of relative toxicity and mutagenicity potential of 1-nitroso-3,5-dinitro-1,3,5-triazacylcohexane (mononitroso-RDX) (Final Report ed.). (ADA149351. USAEHA-75-51-0345-85). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA149351

Stamou, M; Streifel, KM; Goines, PE; Lein, PJ. (2013). Neuronal connectivity as a convergent target of gene × environment interactions that confer risk for Autism Spectrum Disorders. *Neurotoxicol Teratol* 36:3-16.

Suzuki, N; Withers HR. (1978). Exponential decrease during aging and random lifetime of mouse spermatogonial stem cells. *Science* 202: 1214-1215.

Suwa, T; Nyska,A; Peckham, JC; Hailey, JR; Mahler, JF; Haseman, JK; Maronpot, RR..(2001). A retrospective analysis of background lesions and tissue accountability for male accessory sex organs in Fischer-344 rats. *Toxicol Pathol*. 29:467-78. PubMed PMID: 11560252.

Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review] *Regul Toxicol Pharmacol* 62: 107-114. http://dx.doi.org/10.1016/j.yrtph.2011.12.007

Sweeney, LM; Okolica, MR; Gut, CP;, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Regul Toxicol Pharmacol* 64: 205-224.

Takahashi, K; Maejima, H; Ikuta, G; Mani, H; Asaka, T. (2017). Exercise combined with low-level $GABA_A$ receptor inhibition up-regulates the expression of neurotrophins in the motor cortex. *Neuroscience Letters* 636:101-107.

Tan, EL; Ho, CH; Griest, WH; Tyndall, RL. (1992). Mutagenicity of trinitrotoluene and its metabolites formed during composting. *J Toxicol Environ Health A* 36: 165-175. http://dx.doi.org/10.1080/15287399209531632

Testud, F; Glanclaude, JM; Descotes, I. (1996). Acute hexogen poisoning after occupational exposure. *J Toxicol Clin Toxicol* 34: 109-111. http://dx.doi.org/10/3109/15563659609020244

U.S.EPA (Environmental Protection Agency). (1998a). Health effects test guidelines: OPPTS 870.3700. Prenatal Developmental Toxicity Study. Washington, DC, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (EPA/712/C- 98/207)

U.S.EPA (Environmental Protection Agency). (1998b). Health effects test guidelines: OPPTS 870.7800. Immunotoxicity. Washington, DC, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (EPA/712/C- 98/351; http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm)

U.S. EPA (Environmental Protection Agency) (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC; U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (Environmental Protection Agency). (2005). Guidelines for Carcinogen Risk Assessment. (EPA/630/P-03/0001F). Washington, DC; U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (Environmental Protection Agency). (2011). Recommended use of body weight$^{3/4}$ as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC. U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (2012a). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC. U.S. Environmental Protection Agency. Risk Assessment Forum.

U.S. EPA (2012b). Peer review summary report. External letter peer review of study reports on 1,3,5-trinitro-1,3,5-hexahydrotriazine (RDX). Washington, DC. U.S. Environmental Protection Agency.

Von Oettingen, WF; Donahue, DD; Yagoda, H; Monaco, AR; Harris, MR. (1949). Toxicity and potential dangers of cyclotrimethylenetrinitramine (RDX). *J Ind Hyg Toxicol* 31: 21-31.

Wang, C; Leung, A; Sinhia-Hikim, AP. (1993). Reproductive aging in the male Brown-Norway rat: A model for the human. *Endocrinology* 133: 2773-2781.

Ward, JM; Goodman, DG; Squire, RA; Chu, KC; Linhart, MS (1978). Neoplastic and nonneoplastic lesions in aging (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* 83; 849-854.

Watanabe, M; Maemura, K; Oki, K; Shiraishi, N; Shibayama, Y; Katsu, K. (2006). Gamma-aminobutyric acid (GABA) and cell proliferation: focus on cancer cells. *Histol Histopathol* 21: 1135-1141.

West, RR; Stafford, DA. (1997) Occupational exposures and haematological abnormalities among ordnance factory workers: A case control study. *Leuk Res* 21**:** 675-680.

White, H.S.; Franklin, MR; Kupferberg, HJ; Schmutz, M; Stables, J P; Wolf, H H. (2008), The anticonvulsant profile of rufinamide (CGP 33101) in rodent seizure models. *Epilepsia*, 49: 1213–1220. doi:10.1111/j.1528-1167.2008.01552.x

WHO (World Health Organization). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Williams, LR; Bannon, DI. (2009). Mechanism of RDX-induced seizures in rats (Toxicology Study No. 87-XE-OBT9-09). (ADA515072. USACHPPM-TSN-87-XE-OBT9-09). Aberdeen Proving Ground, MD: US Army Center for Health Promotion and Preventive Medicine, Health Effects Research Program. http://oai.dtic.mil/oai/oai?verb=getRecords&metadataPrefix=html&identifier=ADA511072

Williams, LR; Wong, K; Stewart, A; Suciu C; Gaikwad, S; Wu, N; DiLeo, J; Grossman, L; Cachat, J; Hart, P; Kalueff, AV. (2012). Behavioral and physiological effects of RDX on adult zebrafish. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology* 155:33-38.

Williams, LR; Aroniadou-Anderjaska, V; Qashu, F; Finne, H; Pidoplichko, V; Bannon, DI; Braga, MF.(2011). RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RD**X**-induced seizures. *Environ Health Perspect*. 119: 357-363.

.Woody, RC; Kearns, GL; Brewster, MA; Turley, CP; Sharp, GB; Lake, RS. (1986). The neurotoxicity of cyclotriethylenetrinitramine (RDX) in a child: A clinical and pharmacokinetic evaluation. *Clin. Toxicol* 24**:** 305-319.

Wyllie, JL; Devinsky, O. (2015). Epileptic encephalopathies. Optimizing seizure control and developmental outcome. *Epilepsia* 56: 1486-1489.

Young, SZ; Bordey, A. (2009). GABA's control of stem and cancer cell proliferation in adult neural and peripheral niches. *Physiology* 24: 171-185.

Zhang, X; Ebata, KT; Robaire, B; Nagano, MC. (2006). Aging of male germ line stem cells in mice. *Biol Reprod* 74: 119–124.

Zhang, B; Pan, X. (2009). RDX Induces Aberrant Expression of MicroRNAs in Mouse Brain and Liver. *Env Health Perspect* 117: 231-240.

Zuloaga, DG; Lahvis, GP; Mills, B; Pearce, HL; Turner, J; Raber, J. (2016). Fetal domoic acid exposure affects lateral amygdala neurons, diminishes social investigation and alters sensory-motor gating. *Neurotoxicology* 53:132-140.

APPENDIX A: EPA'S CHARGE QUESTIONS

Charge to the Science Advisory Board for the IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) September 2016 (Updated November 2016[1])

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of a draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

IRIS is a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemicals in the environment. Through IRIS, EPA provides high quality science-based human health assessments to support the Agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemicals that can be used to support hazard identification and dose-response assessment, two of the four steps in the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for health effects (including cancer and effects other than cancer) resulting from chronic exposure. IRIS toxicity values may be combined with exposure information to characterize public health risks of chemicals; this risk characterization information can then be used to support risk management decisions.

An existing assessment for RDX includes a reference dose (RfD) posted on the IRIS database in 1988 and OSF and a cancer descriptor posted in 1990. The IRIS Program is conducting a reassessment of RDX. The draft Toxicological Review of RDX is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to RDX. Additionally, appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (see Appendices A to D) to the draft Toxicological Review.

The draft assessment was developed according to guidelines and technical reports published by EPA (see *Preamble*), and contains both qualitative and quantitative characterizations of the human health hazards for RDX, including a cancer descriptor of the chemical's human carcinogenic potential, a noncancer toxicity value for chronic oral exposure (RfD), and a cancer risk estimate for oral exposure.

Charge questions on the draft Toxicological Review of RDX

1. **Literature search/study selection and evaluation.** The section on *Literature Search Strategy/ Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

2. **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al. 2012a; Sweeney et al. 2012b).

   2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

   2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

   2c. In Section 2.1.3 of the draft assessment, an UF of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

3. **Hazard identification and dose–response assessment[1].** In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system- specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance) to reach the following conclusions.

[1] [Note: As suggested by the Chemical Assessment Advisory Committee panel that reviewed the draft IRIS assessment of benzo[a]pyrene, the charge questions in this section are organized by health outcome, with a question on each hazard identification followed by questions on the corresponding organ/system-specific toxicity values. This suggestion, however, entails some redundancy, as some questions apply equally to multiple health outcomes.]

3a**.** Nervous system effects

i. **Nervous system hazard** (Sections 1.2.1, 1.3.1)**.** The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

ii. **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

iii. **Points of departure for nervous system endpoints** (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described**?** Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

iv. **Uncertainty factors for nervous system endpoints** (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a UF_D of 3[2].

v. **Nervous system-specific reference dose** (Section 2.1.4). Is the organ/system- specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

[2] Note that the UF_D applies to each of the hazards identified in the toxicological review.

3b. Kidney and other urogenital system effects

i. **Kidney and other urogenital system hazard** (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

ii. **Kidney and other urogenital system-specific toxicity values** (Section 2.1.1). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

iii. **Points of departure for kidney and other urogenital system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described**?** Is the calculation of the HED for this study scientifically supported and clearly described?

iv. **Uncertainty factors for kidney and other urogenital system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

v. **Kidney and other urogenital system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

3c. Developmental and reproductive system effects

i. Developmental and reproductive system hazard (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

ii. Reproductive system-specific toxicity values (Section 2.1.1). Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?

iii. Points of departure for reproductive system endpoints (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

iv. **Uncertainty factors for reproductive system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

v. **Reproductive system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

3d. **Other noncancer hazards** (Sections 1.2.4, 1.2.6, 1.3.1). The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision. Are other non-cancer hazard adequately described?

3e. **Cancer**

i. **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

ii. **Cancer-specific toxicity values** (Section 2.3.1). As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

iii. **Points of departure for cancer endpoints** (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

4. **Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/basic-information-about-integrated-risk- information-system#guidance) in the following analyses.

4a. **Oral reference dose for effects other than cancer** (Sections 2.1.5–2.1.8). The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

4b. **Inhalation reference concentration for effects other than cancer** (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

4c. **Oral slope factor for cancer** (Section 2.3.3–2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

4d. **Inhalation unit risk for cancer** (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

5. **Executive Summary**. Does the executive summary clearly and adequately present the major conclusions of the assessment?

References

Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-C-8027). Kansas City, MO: Midwest Research Institute.

Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Sub-chronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM. (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report--phase V. Chicago, IL: IIT Research Institute.

Lish, PM; Levine, BS; Furedi, EM; Sagartz, JM; Rac, VS. (1984). Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes1-3). (ADA181766. DAMD17-79-C-9161). Fort Detrick, Frederick, MD: U.S. Army Medical Research and Development Command. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA181766

Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review]. Regul Toxicol Pharmacol 62: 107-114. http://dx.doi.org/10.1016/j.yrtph.2011.12.007

Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64: 205-224. http://dx.doi.org/10.1016/j.yrtph.2012.07.005

APPENDIX B: EDITORIAL COMMENTS

Specific comments on text and presentation of data on reproductive and developmental toxicity

Page 1-39, line 30: Reference to historic controls should be deleted. It is not valid to compare with historical controls (Ward et al. 1979) because it was done by different investigators and no quantitative level of what constitutes testicular degeneration is presented.

Page 1-39, line 33: Add "and a 14% decrease in testis weight" It is useful to add this because this was the only study in which testis weight showed a corresponding decrease with germ cell degeneration, as would be expected.

Page 1-40, line 4: Delete text and references about increases in testis weights. The only significant increase was for relative testis weight and this was really due to a loss of body weight.

Page 1-40, lines 8-21: The presentation and discussion of the results of the two-generation and dominant lethal studies should be combined. They were not separate studies; in fact the same male rats were used for both. Moreover, they show that same result: decrease in yield of pregnancies from males exposed to 50 mg/kg-day. The only difference was the treatment of the females.

Specific comments on presentation of data (Tables, Figures) on reproductive and developmental toxicity

1. The Tables and Figures are well planned and show the important features that need to be presented. However SAB concludes that Table 1 of this report should be added as it compares all the rodent studies in one table, facilitating comparisons showing support and discrepancy.
2. In Table 1-9, the relative testes weights should be deleted. Relative testis weight is affected by changes in body weights, which in our experience does not have effects on testis weights of adult animals. Absolute testis weights are a better measure of testicular toxicity of an agent. The relative testis weights just clutter up the table and add little information on the toxicity of RDX.
3. Table 1-9, Page 1-42: In the presentation of the data of Levine et al. (1983), the data on "SDMS" (spontaneous death or moribund sacrifice) rats should be deleted. Their significance is open to question and they aren't given much weight in the discussion.
4. Table 1-9, Page 1-44: The data on incidence of germ degeneration of Levine et al. (1981a,b, 1990) at 12 and 15 mg/kg-day should be deleted. These were observed on dead rats (all rats in these groups died). Incidentally the numbers were reversed: the value for 1/10 was for the 12 mg/kg-day dose and 1/9 was for 15 mg/kg-day.
5. Table 1-9 (footnote, Page 1-44) Also reference to historic controls for comparison of testicular degeneration reported by Lish et al. (1984) should be deleted.
6. The testis weight data from Cholakis et al. (1980) (Table 1-9, last entry on Page 1-43) on F2 weanlings does not belong in the male reproductive effect section. It is not indicative of direct effect on testis weight and there is no follow-up to determine whether or not adult testis weights will be affected. Rather it belongs in the developmental effects section (Table 1-10).
7. Figure 1-3 could provide a useful comparison of doses from various studies. However, to achieve maximum impact, the data should be grouped as follows: mouse; rat 2-year chronic; rat 13-week subchronic. The study using gavage should be noted since the effective dose seems to be dependent on method of oral administration. Footnote (1), indicating that the non-significant

change in testis weight in Hart et al. (1976) was a slight increase, was confusing and should be deleted; anyway that is covered by the symbol that there was no significant change.

8. Figure 1-3. Additionally, it may be a matter of rote procedure, but the decision to highlight only statistically significant findings in the exposure-response array is deceptive because the two studies identified with statistical significant findings (Levine 1990 and 1983) were not considered meaningful results, but the nonstatistically significant finding in Lish et al. (1984) is the finding for male reproductive effects that is debated heavily in this document and is not highlighted. Perhaps add an explanation via footnotes.

9. Table 1-10 Page 1-46: Reconsider the use of term 'offspring survival' to categorize 'prenatal mortality' as offspring survival is more commonly associated with postnatal outcome.

10. Figure1-4: typo in spelling of 'significantly' in the key.

APPENDIX C: SUGGESTIONS ON THE FORMAT FOR EPA's CHARGE QUESTIONS

The CAAC-RDX panel has the following observations on the charge questions based on experience during the review meeting:

1) Charge questions on the calculation of points of departure for organ/system-specific reference dose did not account for the possibility that the panel may not agree with the selection of the specific endpoint for derivation of a POD (as is the case for the use of suppuratitive prostatitis for derivation of a POD for kidney and other urogenital system effects, and the use of testicular degeneration for the derivation of a POD for male reproductive effect).

Suggestions

- There should be a question if the panel agrees with the selection of a specific endpoint for derivation of a POD, before the question if the calculation of the POD is scientifically supported and clearly described.
- There should also be a question on whether there is an alternative approach.