EPA's Response to Major Interagency Comments on the Interagency Science Consultation Draft of the IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

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Purpose: The Integrated Risk Information System (IRIS) assessment development process of May 2009 includes two steps (Step 3 and 6b) where the Executive Office of the President and other federal agencies can comment on draft assessments. Comments on the Interagency Science Consultation draft of the IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Step 3) were provided by the Council on Environmental Quality (CEQ), Department of Defense (DoD), Department of Energy (DOE), National Aeronautics and Space Administration (NASA), National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), and jointly by the Office of Management and Budget (OMB) and the Office of Science and Technology Policy (OSTP). The following are EPA's responses to major interagency comments. All interagency comments were taken into consideration in revising the draft assessment prior to release for public comment (Step 4a).

For a complete description of the IRIS process, including Interagency Science Consultation, visit the IRIS website at www.epa.gov/iris.

Major Interagency Science Consultation Comments and Responses:

Topic #1: Relationship between convulsions and mortality – *DoD considered EPA's characterization of the relationship between RDX-induced mortality and convulsions (i.e., that increased mortality was generally observed at RDX doses that induced nervous system effects) to be misleading, and suggested that mortality be considered independent of convulsions (possibly as a separate hazard section in the Toxicological Review).*

> **EPA Response:** Characterization of the relationship between mortality and convulsions was based on several studies that reported that convulsions were often observed before unscheduled deaths (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Cholakis et al., 1980). In addition, treatment-related mortality was observed in several studies at doses as low as those associated with nervous system effects (Crouse et al., 2006; Angerhofer et al., 1986; Levine et al., 1983; Levine et al., 1981; Cholakis et al., 1980; von Oettingen et al., 1949). The 90-day DoD-sponsored study by Crouse et al. (2006) provides the most detailed information on the relationship between convulsions and mortality. Additional individual animal data from this study provided by DoD (Johnson, 2015) did not show a clear correspondence between convulsions and mortality in all cases (e.g., not all animals that convulsed died during the study). The Toxicological Review was revised to

better capture the relationship between mortality and convulsions and, in particular, the individual animal findings from Crouse et al. (2006).

Mortality was not presented in a hazard section by itself due to the likelihood that events leading to mortality fall under other specific hazards; however, mortality was discussed in the context of other hazards (in particular, nervous system and kidney effects). In addition, a synthesis of the evidence for RDX-related mortality, including a mortality evidence table, was added to Appendix C. Because some studies identified mortality at the same RDX dose that induced nervous system effects, additional analysis of the mortality data was undertaken. This analysis involved comparison of dose-response relationships for mortality in rodents exposed to RDX for durations up to 90 days with dose-response relationships for convulsions following similar exposure durations. Specifically, LD_{01} values (the dose expected to be lethal to 1% of the animals) derived using mortality data sets were compared to BMD_{01} values for convulsions. In general, this comparison (added to Chapter 2, Section 2.1) indicated that reference values derived from mortality data would be similar to the RfD for RDX based on convulsions, assuming the application of the same extrapolation procedures and uncertainty factors.

Additionally, based on the comments provided by DoD, a question pertaining to the treatment of mortality data was added to the charge to peer reviewers.

Topic #2: Effect of diet versus gavage administration on the dose associated with convulsions – *DoD pointed out that the lower effect level for convulsions in gavage studies (including the 90-day Crouse et al. (2006) study) compared to feeding studies is likely due to a bolus effect (i.e., sudden peak blood and brain concentrations of RDX after dosing), and asked that EPA provide additional discussion of the negative evidence for convulsions in RDX feeding studies.*

DoD also recommended that EPA consider the no-observed-adverse-effect level (NOAEL) from a chronic dietary study in the rat (Levine et al., 1983) as a point of departure (POD) for deriving the RfD, noting that lack of incidence data and uncertainty associated with identification of a NOAEL were overstated as reasons for excluding data from Levine et al. (1983) as a possible basis for the RfD.

DoD also recommended that EPA either use a different dose metric with gavage data, not use the data from the Crouse et al. (2006) study for dose-response analysis, or justify using Crouse et al. (2006) as the key study.

EPA Response: All evidence from diet and gavage studies, both positive and negative, was considered in synthesizing the evidence for convulsions in Section 1.2.1, Nervous System Effects. Specific consideration was given to the differences in convulsion response following gavage and dietary studies in Section 1.2.1. In addition, these differences in response were addressed in the discussion of uncertainties in the derivation of the RfD (Section 2.1) and identified as a key issue in the Executive Summary.

The NOAEL for convulsions from the 2-year dietary study in the rat (Levine et al., 1983) was added to the data sets carried forward for quantitative analysis, and a candidate reference value based on this dietary data set was derived; however, the Levine et al. (1983) study was not selected as the basis for the nervous system-specific toxicity value. The POD based on convulsion data from Levine et al. (1983) was 14-fold higher than the POD derived from Crouse et al. (2006) (selected as the basis for the RfD), and for the following reasons was considered more uncertain than that derived from Crouse et al. (2006). Levine et al. (1983) used four dose groups (plus control) and reported that convulsions and other nervous system effects were observed in rats exposed to RDX for 2 years at the highest dose tested (40 mg/kg-day), but did not report the incidence of nervous system effects. Given the lack of incidence data, BMD analysis was not supported (i.e., the POD was based on a NOAEL). As discussed in the Toxicological Review (Section 2.1.4), daily observations in the Levine et al. (1983) study may not have been sufficiently frequent (animals were observed once daily in the morning) to provide an accurate measure of the occurrence of nervous system effects, potentially leading to underestimation of convulsions. By contrast, Crouse et al. (2006) used five closely-spaced dose groups (plus control) that provided a good characterization of the dose-response curve for convulsions and was specifically designed to assess the nervous system effects of RDX. Justification for using data from Crouse et al. (2006) to derive the nervous system-specific toxicity value was clarified. In addition, a question pertaining to the appropriateness of considering the Crouse et al. (2006) study, which used gavage administration, for developing the nervous system-specific toxicity value was added to the charge to peer reviewers.

As described in Appendix C.1.5, different dose metrics were in fact used with data from gavage and dietary studies. A question pertaining to the choice of dose metric was included in the charge to peer reviewers.

Topic #3: Benchmark response (BMR) of 1% excess risk – *OMB commented that better support is needed for a BMR of 1% extra risk (ER). DoD disagreed with the severity of an endpoint as a criterion for changing the BMR, stating that a 1% BMR is counter to EPA's BMD guidance (U.S. EPA, 2012). DoD also disagreed with extrapolation below the data because it assumes that there is no threshold for convulsions (despite experiments that show doses with no convulsions) and because of the increased uncertainty in extrapolating below the experimental range. Both DoD and OMB suggested the presentation of PODs based on alternative BMRs (e.g., 10% ER) to allow the public and peer reviewers to determine the quantitative effect of EPA's decision and as a measure of uncertainty in the estimated RfD.*

EPA Response: As noted in EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012), the calculation of a BMD is directly determined by the selection of the BMR; selecting BMRs

involves making judgments about the statistical and biological characteristics of the dataset. A BMR of 10% ER is not a default; a lower (or sometimes higher) BMR is often used based on statistical and biological considerations (U.S. EPA, 2012). In the case of RDX, EPA considered the use of a BMR of 1% ER to be justified for an endpoint as severe as convulsions, and consistent with EPA guidance (U.S. EPA, 2012).

Concerning a threshold for convulsions, in vivo animal studies are typically too small to observe low responses, not to mention thresholds. Confidence intervals provide some sense of the uncertainty involved: the upper 95% confidence limit on a 0% response in a group of 20 animals is 14%, and in a group of 50 animals it is 6%. The models in BMDS (EPA's Benchmark Dose Software) that were fit to the data sets showing convulsions (Table 2-2 of the Toxicological Review) were consistent with the observed responses and associated statistical uncertainty. Extrapolation to a BMR of 1% ER does not preclude there being a threshold, since a threshold would involve a BMR closer to 0%.

Uncertainties associated with the use of a 1% BMR were explored as part of the selection of the BMR for nervous system effects (Section 2.1) and identified as a key issue in the Executive Summary of the Toxicological Review. The BMD of 3.0 mg/kg-day was not far below the experimental dose range (4–15 mg/kg-day) in the 90-day Crouse et al. (2006) study (i.e., the basis for the RfD); this extrapolation was therefore considered moderate. A BMR of 1% ER did not result in substantial model uncertainty; BMDLs ranged from 0.54–2.90 mg/kg-day, a 5.4-fold difference, which is also not considered large.

PODs based on alternative BMRs of 1, 5, and 10% ER were presented in the BMD modeling appendix; however, to avoid confusion over the selected BMR, multiple PODs for a single data set were not presented in the body of the Toxicological Review. Reference to the BMD appendix (including specific sections and table numbers) for BMD modeling documentation based on alternative BMRs was provided in the Toxicological Review.

A question pertaining to the use of a BMR of 1% ER was included in the charge to peer reviewers.

Topic #4: Database uncertainty factor (UF) – *DoD did not consider the database UF of 3 (for additional systematic evaluation of neurobehavioral effects) to be sufficiently justified and recommended a value of 1. DoD noted that the 1988 IRIS assessment assigned high confidence to the database and did not include a UF for inadequate database. DoD proposed a charge question on the database UF.*

EPA Response: A database UF of 3 was retained, but the justification in Section 2.1.3 was revised to better support the application of this UF. In particular, EPA identified uncertainty associated with characterization of RDX neurotoxicity, including limitations in study design to assess neurotoxicity, the frequency of animal observations in the available studies that

raises concern of potential underreporting of the true incidence of convulsions, failure to report severity of convulsions and other nervous system effects at the time of observation, and lack of follow-up studies that employed more sensitive assays to assess more subtle neurotoxicity. EPA also notes that the Agency did not start using database UFs until 1994, when consideration of this aspect of uncertainty became routine and more systematic.

A question pertaining to the scientific rationale for the application of a database UF of 3 was included in the charge to peer reviewers.

Topic #5: Cancer descriptor – *DoD commented that the cancer weight of evidence should be reconsidered and a charge question added to ask if the tumor findings and negative genotoxicity data support a finding that RDX is unlikely to be carcinogenic.*

EPA Response: EPA's cancer guidelines include a descriptor of *not likely to be carcinogenic to humans*, a descriptor that is appropriate only when the available data are considered robust for deciding that there is no basis for human hazard concern. In light of the dose-related increases in benign and malignant tumors in the liver and lung of mice and in the liver of rats in 2-year dietary studies, EPA disagrees with DoD's recommendation to consider a descriptor for RDX of "unlikely to be carcinogenic." The cancer descriptor was rewritten to more clearly present the lines of evidence that support the cancer descriptors of *likely to be carcinogenic to humans* and *suggestive evidence of carcinogenic potential*. EPA concluded that the carcinogenicity evidence for RDX most appropriately fell within the spectrum of results covered by the descriptor *suggestive evidence of carcinogenic potential*.

A question as to whether the available human, animal, and mechanistic studies support the conclusion that there is *suggestive evidence of carcinogenic potential* for RDX was included in the charge to peer reviewers.

Topic #6: Application of physiologically based pharmacokinetic (PBPK) models for interspecies extrapolation – *PBPK models have been developed for RDX in the rat, mouse, and human. DoD asked for further justification for EPA's decision to reject the mouse model (developed by Sweeney et al. (2012)) and use instead the default approach for extrapolating doses in animals to humans. DoD noted that Sweeney et al. (2012) did a careful mouse pharmacokinetics study of RDX kinetics by the oral route, and published a mouse PBPK model in a peer-reviewed journal.*

DoD also stated that peak blood (or brain) RDX concentration rather than area under the curve (AUC) is the most supported dose metric, especially as applied to gavage data from the 90-day rat study by Crouse et al. (2006), because peak plasma and brain RDX concentrations have been consistently associated with seizure induction and because of evidence that the maximum concentration in blood (C_{max}) is achieved rapidly after an acute oral gavage dose. DoD asked that a charge question be added regarding support for AUC (versus peak blood concentration) as the dose

metric.

EPA Response: EPA evaluated and further developed the PBPK models for extrapolating doses from animals to humans. Discussion of the uncertainties in the mouse model that led to the decision not to use this model was expanded in Section 2.1.2, with the detailed evaluation of the model and its uncertainties in Appendix C. Major uncertainties in the mouse model included the following:

- The mouse model was based on fitting both the absorption and metabolic rate constants to a single set of blood concentration measurements. In this study, the lowest dose at which RDX was detected was 35 mg/kg, an exposure level high enough to manifest some toxicity in the chronic mouse bioassay, and except for measurements from a single animal, all other data points were non-detects or excluded as outliers (Sweeney et al., 2012).
- The type of additional data that increased confidence in the rat and human models (e.g., in vitro measurements of RDX metabolism and RDX elimination data) are not available for mice.
- There were no data to support characterizing the fraction of RDX that is metabolized in the mouse; this is problematic considering evidence that indicates that the role of metabolism in RDX toxicity may differ across species. Given the high sensitivity of the model to the metabolic rate constant, the uncertainty in mouse toxicokinetics significantly decreased confidence in using the mouse PBPK model for predicting mouse blood RDX concentrations.

The assessment was revised to present PODs for the RfD based on AUC and peak blood concentrations[1], as well as PODs based on the default (body weight scaling) approach for interspecies extrapolation. Section 2.1.2 was revised to provide a more thorough explanation of uncertainties associated with using peak (C_{max}) concentrations as the dose metric. Biological evidence (based on mechanistic information on RDX binding to the picrotoxin convulsant site of the gamma-amino butyric acid [GABA] channel) that supports selection of AUC as a dose metric was also added. EPA notes that the human equivalent dose (HED) using C_{max} as the dose metric to convert the rat gavage dose to a human dose is only 1.3-fold higher than that obtained using AUC, but is associated with greater uncertainty than the HED based on AUC.

A set of questions pertaining to PBPK modeling, including the decision not to use the mouse PBPK model to derive PODs and the choice of dose metric, was included in the charge to peer reviewers.

[1]AUC represents the average blood RDX concentration for the exposure duration normalized to 24 hours. Peak concentration is represented by C_{max}, or the maximum blood RDX concentration for the exposure duration.

References

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