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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.

The literature search that was conducted used a systematic, and unbiased set of search terms with clearly defined inclusion and exclusion criteria to assess primary studies reporting on the primary toxicological outcomes of RDX to animals and humans, including clinical case reports. Several strength of the literature search include its comprehensiveness and the use of PECO statements for delineation of differences in study designs with respect to population, exposure parameters (such as route, timing and purity of RDX), and relevant comparisons were made across doses and routes. In general, potential hazard outcomes were clearly defined and appropriate.

With respect to nervous system hazards for RDX, there were sufficient data to link RDX exposures to convulsions but insufficient information on which guide conclusions about more subtle neurological and behavioral outcomes from sub-convulsive exposures. Moreover, there was a lack of definitive data on the potential influences on neurological and behavioral outcomes after RDX-triggered seizure resolved. Considering that the AOP for RDX is likely due to direct interference with GABA-A receptor functions in the CNS, inclusions of relevant studies on compounds having the same mode of action may have provided additional important clues of the potential hazard of RDX in promoting more subtle neurological (cognitive), behavioral (anxiety) and/or developmental neurotoxicity.

3a. Nervous system effects

(i) **Nervous system hazard.** 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?**

Human Studies

There is consistent evidence from more than 20 clinical case reports that exposure to RDX is associated with adverse neurological outcomes, particularly an association with convulsions. Never the less, there are many and varied limitations to concluding about the hazards of RDX

solely based on such case reports. There is only one cross-sectional study that provides a snapshot of the potential neurotoxicity associated with pulmonary exposure to RDX. Ma and Li (1993) presented results from a number of neurobehavioral functioning and memory tests from a study of workers exposed in a Chinese RDX plant at a single point in time. The results indicated significant neurobehavioral and memory deficits were associated with RDX exposure measured in air. However, this study has several significant limitations that should limit conclusions about RDX hazard solely based on its findings. Of greatest concern was the omission of exposure levels in the “non-exposed” group, no attempt to control for confounders (non-occupational exposures, lifestyle confounders, influences of co-morbidity factors), and the lack of a rationale for subdividing the exposed cohort. Nevertheless, the outcomes on Composite Memory Retention Quotient and Composite Block Design score were greater >15 points and ≥ 2 sec lower ($p < 0.01$) than the control group, respectively. Statistical analyses seem appropriate but 95% CIs would have been helpful since 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% SD.

Animal Studies

Several studies with rodents using oral gavage and dietary exposure models over the acute, sub-chronic and chronic timeframes have consistently identified a broad range of neurological impairments ranging in severity. Convulsive (seizure) activity is a common in most, but not all, studies. In addition to seizure activity, several of these studies identified “less severe” neurological and behavioral impairments that may be consistent with the sparser literature on human exposures. An important observation stemming from some of the animal studies is that RDX appears to sensitize animals exposed to lower doses to subsequent seizurogenic stimuli, including electrogenic, audiogenic, and chemical kindling. Another potentially important experimental finding is that exposure dose appears to be a more important predictor of adverse neurologic outcomes than is duration of treatment.

Mechanistic Studies

The neurotoxicity profile of RDX is consistent with that of a centrally acting excitotoxicant. There is ample direct evidence of a direct interaction of RDX with GABA-A receptors (GABR) in the mammalian CNS. RDX blocks GABA activated Cl^- currents and the inhibitory postsynaptic potentials (iPSPs) that form critical inhibitory networks throughout the brain. The available data also suggest that the limbic system, including the amygdala, is especially sensitive targets of RDX. Surprisingly, the potency of RDX as a GABR blocker is relatively low. By comparison picrotoxin (PTX) has >100-fold lower K_i (100x more potent) than RDX at binding to

GABR (K_i 0.2 vs 21 μM). The lower potency of RDX extends to the concentrations needed to inhibit Cl^- currents in whole cell voltage clamp experiments and iPSC events, which typically require $\geq 10\mu\text{M}$. Also relevant to RDX mechanism and its potential importance to long-term behavioral toxicity is the observation that the inhibitory actions of RDX on seizure-like neuronal discharges measured in the basolateral nucleus of amygdala.

Preliminary Conclusions

Despite the limitations of the Ma and Li (1993) study, considered with the sum of evidence from clinical case reports, results from experimental animals, and mechanistic studies on RDX, there is necessary and sufficient evidence to support the conclusion that nervous system toxicity is a human hazard of RDX exposure. With respect to whether all hazards to the nervous system have been adequately assessed, the measure of abnormal electrographic activity or seizure-like activity in specific brain regions may be a more sensitive indicator of neurotoxicity and the potential of RDX to elicit more subtle neurological impairments such as cognitive deficits and/or behavioral abnormalities.

(ii) **Nervous system-specific toxicity values.** 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 are scientifically supported, clearly described, and sufficient to draw conclusions about the potential hazards associated with exposures to RDX. The differences in toxicokinetics of RDX exposure by gavage vs. dietary administration is clear, and must be accounted for when predicting risk. Based on the state of the science, it appears that the gavage route for RDX results in high peak blood and brain levels than the dietary route. Also the rate of rise in blood and brain levels is faster with gavage. Since it is the dose rather than the duration of exposure that is more predictive of neurological outcomes, at least from animal studies, it is appropriate to consider the dose–response data reported in Crouse et al. (2006) study as a relevant model that should be protective of dietary and dermal exposure paradigms. However, it is not likely that the results from Crouse et al 2006 would be protective of pulmonary exposures which may lead to faster and higher blood/brain levels at equal dose. There remain several unanswered questions (uncertainties) with regard to the dose-response

relationships leading from seizure to convulsive activity and lethality. Based on the available data, death may occur without seizure of convulsions detected, although this may simply be due to the lack of adequate (sufficiently frequent) observations by the experimenters. However, based on the current state of the science (including the epilepsy literature), death is not a necessary outcome of seizures of convulsions, and is driven by abnormal electrographic patterns in the brain. It is therefore reasonable to conclude that convulsion as characterized in the report is a reasonable severe endpoint for human risk assessment.

(iii) Points of departure for nervous system endpoints. 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?

The selection of convulsions as the endpoint to represent the most severe endpoint for nervous system hazard is scientifically supported and clearly described in the document. Several, more subtle cognitive and behavioral neurological impairments are not considered and integrated into the report per se, although there is strong evidence from several clinical reports, the cross-sectional study of Ma and Li (1993), and a number of rodent studies that they can occur without evidence of convulsion. This raises the question of whether calculations of PODs and HEDs, although scientifically supported and clearly described in the document, are in fact protective of more neurological impairments in the absence of convulsion. Never the less, several factors mitigate this concern: (1) the intrinsic potency of RDX for binding GABR is >100-fold lower than that of a comparable ligand (PTX) with the same AOP; (2) the acute dose required for eliciting convulsions is >100-fold higher than that required for PTX; (3) the indication that dose rather than exposure duration is a more important indicator of adverse outcomes, and (4) evidence from animal studies of other seizurogenic compounds with similar AOP that indicate the LOELs for triggering abnormal electrographic patterns and those that produce convulsions are within a factor of 2-3 fold dose range.

Based on the sum of the data available, it is concluded that the severity of convulsions warrant the use of a benchmark response level of 1% extra risk. Base on the available science, use of the BMDL for convulsion is considered appropriate and consistent with the EPA's Benchmark Dose Guidance.

(iv) Uncertainty factors for nervous system endpoints. 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 3.2

The POD for neurological effects is based on the most detailed D-R data available. The gavage route of exposure should be protective of the more human-relevant dietary route because the former yields more rapid distribution and higher blood levels than the latter. However, whether the uncertainty factors will be protective of pulmonary exposure to fine RDX dust remains uncertain. Another uncertainty is whether differences in human susceptibility ($UF_H = 10$) is defensible given the growing number of highly penetrant gene variants associated with genes that encode human GABR subunits that confer and/or enhance susceptibility to not only seizure disorders but also behavioral impairments.

(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?

Based on the available data, the nervous system RfD is supported by the available scientific literature and clearly presented.

Executive Summary

The executive summary is for neurological effects of RDX is presented in a clear and concise manner. It accurately summarizes the strengths and weaknesses of all the reliable data.