

Responses to Charge Questions on Draft Toxicological Review on RDX

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Literature search/study selection and evaluation

In general, the literature search process is well described, thorough, and clearly documented. The search strategy, study selection considerations including exclusion criteria, and evaluation mechanisms are appropriate and well defined. There are no apparent sources of additional peer-reviewed studies that should be considered.

Study results are evaluated using quality considerations that addressed aspects of design, conduct, or reporting that could affect the interpretation of findings, overall contribution to the synthesis of evidence, and determination of hazard potential according to EPA guidelines. The purpose is to identify the stronger, more informative studies based on this uniform evaluation of quality characteristics across investigations of similar design.

Evaluation of the experimental animal database for RDX is limited to studies of oral toxicity. A well defined set of studies provided useful information on RDX toxicity, including an array of endpoints for neurotoxicity and immunotoxicity. A secondary set of studies provided information relevant to RDX toxicokinetics and mechanism of action on the nervous system, but not health effects data.

Hazard Identification – 3a(i). Nervous system hazard

It is clearly apparent that the available research evidence supports the assessment conclusion that nervous system toxicity is a human hazard of RDX exposure. In a cross-sectional study neurobehavioral function was impaired in Chinese workers occupationally exposed to RDX (Ma and Li, 1993). Memory retention and block design scores were significantly lower among exposed workers compared to unexposed workers from the same plant. These types of effects are more sensitive to low levels of exposure than seizures, and are typical of the reported actions of many more common environmental agents. However, this study did not consider potential confounders, and there was limited information characterizing RDX exposure, thus significantly diminishing the value of the findings. Two other human case reports also suggest an association between RDX exposure and neurological effects.

Nervous system effects in experimental animals include a wide array of behavioral changes – irritability, hyperreactivity, tremors, and other signs that may be considered prodromal of convulsions - consistent with the induction of seizures by RDX exposure, and these actions have been observed in the majority of chronic, subchronic, and developmental studies examining oral exposure to RDX. There also is significant evidence from the scientific literature to indicate that RDX neurotoxicity results from interactions of the compound with the GABA_A receptor. These findings are consistent with the ability of RDX exposure to induce convulsions and related

behaviors. While this evidence does not preclude the influence of other receptors as yet unstudied for RDX binding affinity, it does support involvement of the GABAergic pathway described above in the development of the neurotoxicity. In contrast, evidence supporting a role for glutamate in the effects of RDX is very limited, and a basis for excessive glutamate stimulation in the assessment is weak, if not unfounded.

All hazards to the nervous system are adequately assessed, and there is no other more appropriate endpoint to address the spectrum of effects than convulsions, as these behaviors are observed in a significant proportion of the studies.

3a(ii) – Nervous system-specific toxicity values

The criteria utilized for the selection of studies reporting nervous system effects are scientifically sound and clearly described. By reviewing 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 it is apparent to this reviewer that the most reliable scientific information has been accessed for this assessment.

The assessment has identified the data generated by Crouse *et al.* (2006) as a primary basis for judgments about the nervous system effects of RDX. This study and several others in the select group reporting effects on neurological health utilized gavage administration of RDX as opposed to a dietary route of administration. While dietary routes offer a more consistent ingestion and absorption of RDX and are perhaps better suited for chronic exposure studies, gavage procedures provide a more focused and intermittent administration of the compound. It is also true that there is less variability in the amount of the toxic agent delivered than for dietary intake that is dependent on the animal's feeding habits. As long as these characteristics of administration are understood and accounted for, there is no reason to exclude the work using gavage routes of exposure. Moreover, the Crouse *et al.* (2006) study produced perhaps the best RDX dose-response data available.

The most commonly reported nervous system effect of RDX is the induction of convulsions despite their character as severe functional endpoints. While many of these studies noted seizures as an observation, most of them did not track and report incidence or frequency or less severe behavioral signs that would support better analysis of this toxicity. The assessment presents the rationale for ascribing value to the Crouse *et al.* (2006) findings in a clear and logical manner. The potential relationship of convulsions to the mortality observed is also considered in the assessment, resulting in the conclusion from human and animal data that these two outcomes are not linked. The judgments in the assessment that led to these conclusions are appropriately described.

3a(iii) – Points of departure for nervous system endpoints

The most commonly reported nervous system effect of RDX is the induction of convulsions despite their character as severe functional endpoints. While many of these studies noted seizures as an observation, most of them did not track and report incidence or frequency or less severe behavioral signs that would support better analysis of this toxicity. The judgments in the assessment that led to this conclusion are scientifically supported and clearly described.

The calculation of PODs and HEDs for the selected studies are scientifically supported and clearly described. The severity of convulsions as an exposure outcome reasonably justifies the use of a benchmark response level of 1% extra risk. This value should be conservatively established, as the data on convulsions induced by RDX has limitations, particularly with respect to milder signs of enhanced neuronal activity that may precede seizures. Calculation of the lower bound on the benchmark dose for convulsions is appropriate and consistent with the EPA's guidance.

3a(v) – Nervous system-specific reference dose

The nervous system-specific reference dose for RDX is scientifically supported, conservatively established, and clearly characterized. The reference dose for the nervous system is very close to the value for the kidney/urogenital system, lending additional credence to both values. The nervous system dose is about an order of magnitude lower than that for the male reproductive system, but merits the highest confidence level of the three values and was also selected as the overall reference dose. The assessment considers the confidence levels for the non-nervous system doses to be low.

4a – Oral reference dose for effects other than cancer

The selection of the overall reference dose for RDX based on the nervous system effects described in Crouse *et al.* (2006) is scientifically sound, clearly described, and conservatively determined. This conclusion also takes into consideration mortality as well as the lower organ system-specific reference dose reported by Cholakis *et al.* (1980) – the link of convulsions and mortality is weak, if not non-existent, and the study by Cholakis *et al.* (1980) has greater limitations than that of Crouse *et al.* (2006).

Specifically, Cholakis *et al.* (1980) was designed as a developmental toxicity study with only routine monitoring of clinical signs. This study used only three dose groups with order of magnitude spacing, resulting in a less reliable characterization of the dose-response curve for convulsions than by Crouse *et al.* (2006). The Cholakis *et al.* (1980) study was also of much shorter duration and thus is less suitable for extrapolation to chronic exposure.

Executive summary

The executive summary presents a lucid and succinct description of the major conclusions of the assessment. This includes a compilation of the neurological signs, an explanation of the basis for

the overall reference dose, a justification for exclusion of inhalation data, and a brief description of mechanistic evidence.