Comments to the EPA Science Advisory Board Review Meeting for RDX
Submitted by the Office of the Secretary of Defense, Department of Defense
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The Department of Defense (DoD) appreciates the discussion during the public peer review (teleconference) meetings on April 13 and 17, 2017, for the Science Advisory Board-Chemical Assessment Advisory Committee (SAB-CAAC) on the review of EPA's draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). The DoD provided written comments and participated in the discussion and, as a result, we remain concerned about the ongoing SAB-CAAC recommendation to use an observation from only one animal that was observed in one study (Cholakis 1986) as the basis for determining the reference dose, especially when several important issues have already been raised regarding the quality of that study. The DoD would like to note that the EPA voiced similar concerns with use of the Cholakis study in their opening remarks to the SAB-CAAC for RDX, and we agree with their points. We also agree with EPA's position, as described in opening remarks, that a database uncertainty factor (UF_D) applied to the oral reference dose of 3 is sufficient to account for uncertainties and should not be raised to 10.

The DoD appreciates the opportunity to provide additional comments for consideration by the SAB-CAAC as they finalize their reviews and findings for the EPA. We offer the following input for your consideration.

- The Cholakis (1986) study used test material that was 9% octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). The potential for interactions, including synergy, for the combination is unknown. Furthermore, the reported exposure of 2 mg/kg-d is based on a calculated (nominal) dose that, based on their analytical chemistry data, could have varied widely of that value. No demonstration of homogeneity or stability of test compound in the vehicle was provided. The selection of a low quality study that was rejected for use by the EPA in the previous assessment seems difficult to justify when there are currently higher quality studies that provide good dose-response information. Selecting the study with the lowest response, regardless of quality or statistical assessment is contrary to the current practice of IRIS toxicity evaluations, as is reflected in its current draft for RDX. We support the current IRIS practice of evaluating the quality of studies as well as their results.

  Furthermore, the DoD suggests that selection of the Cholakis study is not supported by the additional information now available with respect to doses that would elicit seizures (Williams *et al*., 2012). RDX levels in brain that result in seizures have been reasonably well established (8 µg/g from the Williams *et al.* study; higher for other studies). The K_i of RDX for the GABAA receptor is 20.1 µM, which translates to a value of 4.5 µg/mL in brain, at which 50% of GABAA receptors would be bound by RDX. Rats given a single dose of 3 mg/kg RDX showed peak values of 2 µg/g in brain at 3.5 hours post dosing (Bannon et al., 2009), which is well below values required to initiate seizures. In addition, this dose was completely eliminated before 24 hours post dosing, indicating that at least 5 plasma half-lives had been exceeded. This dose is close to the $BMDL_{05}$ of 2.66 mg/kg-day suggested for use by the SAB-CAAC. This proposed BMDL dose would be eliminated from the body by the time of next dosing in rats, and there could not be a buildup of RDX in the body over a 90-day subchronic study. Thus, the DoD suggests that the data do not support the conclusion that lower doses could cause either seizures or mortality given what is known about the molecular mechanism of action. This analysis also provides a molecular explanation as to why the carefully controlled and monitored doses of 4 mg/kg-day used in the Crouse study

did not result in seizures or alterations in behavior monitored through the functional observation battery, as well as showing why the doses used by Cholakis et al. (i.e., 2 mg/kg-d) might have likely have produced a response in one animal due to wide differences in the nominal vs the actual doses used.

The DoD appreciated the SAB-CAAC panel expert's discussion of the various ways in which effects at lower exposure levels may be accommodated through the use of EPA's benchmark dose (BMD) software and through the use of uncertainty factors (UFs). Consistent with both the discussion at the teleconferences and with EPA guidance, we want to highlight the following points:

- As discussed by one of the panel members, EPA's Benchmark Dose Technical Guidance recommends that the point of departure (POD) for further analysis be near the low end of the data where effects were observed.

  During the teleconference, one reason for this was provided, i.e., that the ratio of the lower confidence limit (BMDL) to the best estimate (BMD) increases as the benchmark response level (BMR) decreases. This ratio of BMD/BMDL goes from 6-, 2-, and 1-fold for BMRs at 1%, 5%, and 10% respectively. Clearly, the 5% and 10% BMRs are optimal for capturing the variation in response. An additional reason is the recognition that the dose-response function may change with an inflection point between the higher and (extrapolated) lower exposures. While the best known of these is the existence of thresholds, other biological processes, such as competition for binding sites, could conceptually affect the dose-response function. If the effect is observed above such a transition, the shape of the dose-response curve below that effect level is not known.

- One reason to reduce the BMD would be the association between mortality and seizure event in some species. However, this relationship does not appear to be supported by the data (neither animal nor human), as the SAB-CAAC has stated.

- The suggestion that the POD should be lower because of the severity of the effect does not seem to have scientific justification. We assume that EPA, like DoD, would find seizures in < 1% of the population as unacceptable as seizures in < 10% of the population (with a 95% level of confidence).

- If the lower BMR is to protect against effects that were not observed, e.g., effects other than seizures in adults, extrapolation below the observed effect is difficult to scientifically justify. As previously mentioned, less severe adverse effects are unlikely to have the same dose-response function as seizures. In many cases, the dose-response functions are less steep due to more variability in response. It is more likely that, as the effect changes, the dose-response function will also change.

The DoD does not believe that raising the UF_D to 10 would be consistent with practices followed by the EPA IRIS program, which generally uses recommendations in *A Review of the Reference Dose and Reference Concentration Processes* (EPA 2002). That document suggests that a factor

of 3 be applied when a prenatal toxicity study or a two-generation study is missing, and one of 10 applied when both types of studies are missing. In the draft RDX Toxicological Review, EPA notes that both types of studies have been performed on RDX, but chose to apply a UF_D of 3 to account for RDX neurotoxicity and a concern that additional study would find a more sensitive endpoint and lower POD. We agree with EPA's position that raising the UF_D to 10 is not necessary to account for uncertainties in the RDX's database and believe that the UF_D of 3 used more than adequately addresses their concerns relative to neurotoxicity and offer some additional thoughts below.

- As discussed by several of the panel members, other UFs are designated to cover sensitive members of the population, including *in utero* exposures. Where it has been examined, the maximum value of the UF, i.e., 10, has been found to include the more sensitive individuals that have been identified. While the possibility of RDX being an extreme outlier exists, there are at least two ways to deal with these concerns that are discussed below.
    - As discussed in the SAB-CAAC, the effect is considered a frank (or severe) effect. There is usually less inter-individual variability for frank effects.
    - If there is quantitative information on chemicals with the same or similar mode of action, these data can be used to infer on the probability of neurodevelopmental effects and be used to justify a change from the standard default values of a UF (1, 3, or 10) to a numeric value. This is described in in EPA's 2014 *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*.

Thank you for considering the DoD's comments.