

Response to EPA Comments on Study Quality Considerations of Cholakis et al (1980) – by Joanne English

1) study quality considerations (single incident of convulsion in positive control group); 2) use of Cholakis in addition to the data from Crouse et al. (2006).

Although the Cholakis et al. (1980) teratology study is older and the reporting somewhat flawed, the report contains valuable data on the nervous system effects of RDX administered to pregnant F344 rats by gavage. Interestingly, the dose-response data *viz.* incidence of overt neurotoxic signs, reported in Cholakis et al. (1980) is remarkably similar to that of Crouse et al. (2006) in spite of vastly differing dosing durations (14 days in the Cholakis teratology study and 90 days in the Crouse study). This similarity in dose-response relationship between the two studies suggests that the studies are corroborative of each other.

Comment 1 from EPA notes the observation of a single incident of convulsion in the positive control group treated with hydroxyurea (Cholakis et al. 1980), but it is unclear what bearing this has on study quality. In the Cholakis 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 positive control substance, is also known to target the CNS (fetal and adult). Post-marketing experience with patients on hydroxyurea has identified nervous system disorders, including convulsions; thus it is not implausible that hydroxyurea elicited a convulsion in the Cholakis study.

- FDA's Professional Drug Information database indicates that adverse effects associated with use of hydroxyurea based on post-marketing experience include: "*Nervous system disorders*: headache, dizziness, drowsiness, disorientation, hallucinations, and convulsions." Convulsions (seizures) are identified in consumer literature as rare side effects of taking hydroxyurea. <https://www.drugs.com/pro/hydroxyurea.html> Convulsions are identified in product sheets for HYDREA® and DROXIA® (hydroxyurea capsules, USP) as adverse events associated with the use of hydroxyurea in the treatment of neoplastic diseases.
- Such human experience in isolation is difficult to rely on due to potential unexamined confounders, but the IARC monograph on hydroxyurea makes it clear that hydroxyurea targets the central nervous system, at least during development. It is rapidly and widely distributed, including to the cerebrospinal fluid. <https://monographs.iarc.fr/ENG/Monographs/vol76/mono76-14.pdf>

Morton et al. (2015) studied the toxicity of hydroxyurea after repeated oral dosing of rats and dogs. This study was cited as evidence that hydroxyurea does not cause convulsions in laboratory animals. It should be noted, however, that there was other evidence of CNS stimulation (aggression, observed in male rats given 1,500 mg/kg-day hydroxyurea by gavage). There may be more relevant data for assessing the potential of hydroxyurea to cause convulsions in laboratory animals. Morton et al. dosed animals by the oral gavage route, which can be anticipated to result in slower absorption and lower blood and brain concentrations relative to the intraperitoneal route that was used in the Cholakis study. 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. The most appropriate literature to search to examine the evidence of whether hydroxyurea elicits convulsions in laboratory animals is single dose (i.e., not restricted to repeated dose) studies in which pregnant animals are dosed by the intraperitoneal route. EPA apparently limited their literature search to repeated dose studies. A single, i.p. dose of hydroxyurea was used by Cholakis et al. and is a commonly used paradigm for the positive control in teratology studies. It is unclear at this time what a more comprehensive search of the literature and databases relevant to hydroxyurea toxicity would reveal, but absent such information, the Cholakis 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.

An additional quality issue that was raised regarding the Cholakis et al. (1980) study is the homogeneity of the dosing preparations. In both the Cholakis et al. (1980) teratology study and the Crouse et al. (2006) study, doses of RDX were administered in a methyl cellulose / Tween 80 vehicle as a suspension. Cholakis acknowledges that “maintaining uniform suspensions was not always easy,” and when the same nominal concentration was assayed repeatedly, it showed wide variation in RDX content (33% to 500% relative to nominal). Less variability in RDX dose suspensions was reported by Crouse et al. (2006), who report: “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, where both under-dosing and over-dosing of animals is a concern due to the difficulty in maintaining uniform dose suspensions.

Given the quality issues identified for the Cholakis study; some of those articulated in the EPA draft report and the concern described above regarding the high variability of dose levels based on the difficulty in maintaining homogeneous dosing suspensions, it is reasonable to give more weight to the Crouse et al. study with respect to the quantitative dose-response analysis. However, the single incidence of convulsion observed in the 2 mg/kg-day RDX dose group in the Cholakis study cannot be entirely discounted. Pregnant rats were used in the Cholakis study and non pregnant male and female rats were used in the Crouse study, and the available dataset does not resolve the question of whether pregnancy increases susceptibility to RDX. Accordingly, the selected point-of-departure, if based on Crouse et al. (2006), should take into account the empirical observation of convulsion observed at the mid-dose in Cholakis et al. (1980).