

Question #1: Literature search/study selection and evaluation

The literature search and strategy were well done and thorough and included appropriate sources of literature and studies, including the Department of Defense, Defense Technical Information Center (DTIC) database. Inclusion and exclusion criteria were clearly articulated. Considerations included animal species, sex and strain; experimental design; test article source and exposure; endpoint assessments; and data analysis and presentation.

The quality of the health effects data (human and animal studies) was assessed and reported. Two of three reports with human data were determined to be noninformative. Experimental animal studies include 3 chronic 2-year studies in mice and rats; 10 subchronic studies in mice, rats, dogs and monkeys; 1 reproductive study in rats; 4 developmental studies in rats and rabbits; and one acute study of nervous system effects in rats. All, except two studies, were only available as unpublished contract research laboratory reports. Studies in nonstandard species (e.g., deer mice, lizards, quails) were not used to assess health effects, but did provide information relevant to toxicokinetics and mechanism of action (MOA).

Question #3b (i): Kidney and other urogenital system hazard (sections 1.2.2, 1.3.1).

The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

The two-year bioassay in F344 rats had urogenital effects in the male rats consisting of medullary papillary necrosis, pyelitis, (uremic) mineralization, bladder distension and cystitis, and suppurative prostatitis in the high-dose (40 mg/kg/day) group. Suppurative prostatitis was also reported in the mid-dose groups (1.5 and 8.0 mg/kg/day). Male B6C3F1 mice (320 mg/kg/day) had tubular nephrosis in a 90-day study. Male and female monkeys (10 mg/kg/day) had minimal to mild mineralization of the medulla in the 90-day study. No dose-related changes were found in a subchronic study in dogs or rabbits.

The report emphasized the significance of suppurative prostatitis as a surrogate marker for the urogenital effects in F344 rats and indicated potential relevance to humans. This is somewhat misleading. Prostatitis (nonsuppurative and suppurative) is a common background lesion in rats, particularly F344 rats. The report should distinguish between the incidence of nonsuppurative and suppurative prostatitis and determine if there is a relationship between the two types of prostatitis. The report did not specify what lobes of the prostate gland were affected. It is not clear whether all lobes (dorsal, lateral, ventral, and anterior) of the prostate gland were evaluated. The report also states that

the incidence of prostatitis in control rats was low. Suppurative prostatitis in rats is usually due to a low-grade, chronic bacterial infection. Suppurative prostatitis may occur with nonsuppurative prostatitis and both forms of prostatitis can wax and wane in an individual rat. The suppurative prostatitis was associated with the other urogenital lesions (papillary necrosis, mineralization, bladder distension and cystitis, and pyelitis), particularly in the high-dose group. Inflammation of the lower urinary tract and urine retention is a predisposing factor for nonsuppurative and suppurative prostatitis. It is likely that the lower urinary tract changes, particularly bladder distension, urine retention, and cystitis, predisposed to the development of suppurative prostatitis.

Suppurative prostatitis was most frequent in moribund high-dose rats and there were only 4 terminal sacrifice high-dose rats with an incidence of 0/4 for suppurative prostatitis. The urogenital lesions were considered a major contributing factor to the moribund state of the rats. It was emphasized that there was a dose-response relationship in the incidence of suppurative prostatitis. The majority of the rats with suppurative prostatitis in the mid-dose groups were also in moribund animals. It would be useful to know if there were any conclusions on the cause of the moribund state in the mid-dose rats, since the other urogenital lesions were not as frequent as the suppurative prostatitis. It is possible that the mid-dose rats also had urine retention, which was not detected as bladder distension, at the time of necropsy.

Prostatitis (nonsuppurative and suppurative) is a common background lesion in rats, particularly F344 rats. The increased incidence of suppurative prostatitis in the mid-dose and high-dose rats was likely secondary to other urogenital RDX-associated findings that contributed to a moribund state. Prostatitis was not reported in other toxicity studies in rats, mice, dogs and monkeys. It was hypothesized that urine retention may have been secondary to the effects of RDX on the GABA_A receptor in the lower urinary tract in rats. This is plausible, but no mechanistic studies support this contention. It is unlikely that the suppurative prostatitis associated with RDX administration will translate to similar findings in other animal species and men; therefore, it should not be considered a surrogate marker for the other urogenital system findings associated with administration of RDX.

Question #3b (ii): Kidney and other urogenital system-specific toxicity values (section 2.1.1). *Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?*

The use of suppurative prostatitis as a surrogate marker of risk assessment of RDX is not justified (see above). Suppurative prostatitis occurred most frequently in moribund F344 rats in the mid-dose and high-dose groups and was infrequent in terminally sacrifice rats. The suppurative prostatitis was likely secondary to other RDX-mediated effects on the urogenital system, particularly in the high-dose group.

Question n #3b (v): Kidney and other urogenital system-specific reference dose (section 2.1.4). *Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?*

Based on comments in 3.b (i and ii) it does not seem appropriate to calculate an oral reference dose based upon the urogenital system findings in the F344 rat 2-year study.

Question #3e (i): Cancer hazard (sections 1.2.5, 1.3.2). *There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusions.*

The hazard descriptor, 'suggestive evidence of carcinogenic potential' is supported by the animal bioassay data. The incidence of liver tumors (adenomas and carcinomas) was increased in female B6C3F1 mice in a dose-dependent manner and reached statistical significance in some groups (particularly the high-dose group) depending on the analysis. The liver tumor incidence data were variable in male mice and were not convincing for a RDX-mediated effect. A review of the liver tumors in female mice was conducted by a Pathology Working Group (PWG), which provided additional validity to the interpretation and (re)classification of adenomas and carcinomas based on current standards. The lung tumor data in B6C3F1 male and female mice were less supportive of a carcinogenic effect; however, statistically significant increases in tumor incidence were reported for carcinomas or combined adenoma/carcinoma. The data on liver tumors in F344 rats did not support a carcinogenic effect of RDX.

Question #3e (ii): Cancer-specific toxicity values (section 2.3.1). *As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?*

The rationale for quantitative analysis of carcinogen risk analysis was appropriately justified and the selection of *Lish et al (1984)* was supported and clearly described.

Question #4c: Oral slope factor for cancer (sections 2.3.3 – 2.3.4). *The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?*

The lung tumor data is not considered robust, so it may not be advisable to combine the mouse liver and lung tumor data for analysis of the oral slope factor.

Question #5: Executive Summary. *Does the executive summary clearly and adequately present the major conclusions of the assessment?*

There is too much emphasis on the incidence and significance of the suppurative prostatitis. The other urogenital system effects are of more importance and should be described. It does not seem appropriate to calculate a reference dose based on the incidence of suppurative prostatitis.