

Marvin L. Meistrich

3.c.i - **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

A thorough review of the reports (a few publications but mostly unpublished technical reports) is presented.

The conclusion of suggestive evidence of male reproductive effects is based on evidence of testicular degeneration in male mice by Lish et al. (1984). As described in 3.c.ii, there are several weaknesses with this evidence.

On the other hand, Cholakis et al. (1980) shows a reduction (which I calculate are significant) in pregnancy rates at the high dose group when males were treated. I suggest adding a table such as the one suggested below.

Reference and study design	Results					
Pregnancy rates						
Cholakis et al. (1980)	Doses	0	5	16	50†	50†
Dominant Lethal Study (Only males treated)	Incidence	85/88	87/88	79/88 ^a	60/76*	
	Percent	97%	99%	90% ^a	79%*	
F0 of 2-generation study Both parental rats treated	Incidence	17/22	21/22	20/22	11/22 ^b	11/17
	Percent	77%	95%	91%	50% ^b	65%

* Statistically significant ($p < 0.05$); reviewer's calculation

^a Suggestive of statistical significance ($p = 0.07$)

^b Suggestive of statistical significance ($p = 0.06$)

† Presentation of data from 2-generation study was unclear; 22 females tested; 17 females co-housed, uncertain as to what should be the denominator

Examining the pregnancy data from the 2-generation study (Cholakis et al, 1980), it is apparent that the loss of statistical significance of the pregnancies in the 50 mg/kg-day group may be a result of the incidence of pregnancies in the control group being lower than those in the 5 and 16 mg/kg-day groups. Although the conclusions in the draft that these effects may be due to the general toxicity of RDX or a treatment-related decrease in well-being of the males in the high-dose group, they may very well be indicative of reproductive toxicity towards the male and/or the female.

In addition, the effects of RDX on fetal and offspring survival, and offspring development (Cholakis et al, 1980) (Table 1-10, Page 1-46) should be given more emphasis. The survival at weaning (8% in F1 of the 2-generation study and 0% in F2) is highly significantly different from the control values (87% and 79% respectively). Even though there is some toxicity at this dose (18% mortality, ~10% reduced body weights, ~14% reduced food consumption), the effects on fetal and offspring survival are huge and very important, and deserving of more consideration.

3.c.ii - **Reproductive system-specific toxicity values** (Section 2.1.1). Is the selection of the [Lish et al. \(1984\)](#) study that describes male reproductive system effects scientifically supported and clearly described?

The Lish report provides very weak suggestive evidence that the highest doses produce testicular degeneration by histological assessment. There are numerous weaknesses in the results and the presentation.

In the Technical Report and the draft Toxicological Review of RDX (Page 1-39, line 30) reference is made to comparison with historic controls. It is impossible to make such a comparison because it was done by different investigators (Ward et al., 1979) no quantitative level of what constitutes testicular degeneration is presented. The comparisons with historic controls should be deleted.

There are problems in 2-year chronic studies of reproduction in mice and rats. At 2 years of age there is spontaneous deterioration in spermatogenesis (Zhang et al. 2006; Beattie et al. 2015). Significant effects may be due to combined effects of chemical and aging and not a result of the prolonged treatment. This caveat should be mentioned for all the 2-year chronic studies. No treatment induced testicular defects were reported by Lish et al. (1984) for the 6-month or 1-year tissue harvest, indicating that these defects were not present, although fewer mice were analyzed at these times.

There are some questions about the statistical significance of the numbers. Lish did not claim that the incidence of testicular differentiation was not significantly increased from concurrent or historical controls. However, by a simple Fisher exact test, I found that the incidences of degeneration at 100/175 mg/kg-day (3/27) and at 35 mg/kg-day (6/59) were both significantly different from control at $p = 0.025$ and $p = 0.011$, respectively. I suggest that the significance of these results be re-examined with a proper test. If they are not statistically significant, I do not think they should be used for calculation of a Reference Dose.

The suggestion that there was testicular degeneration was weakened by the failure to find any significant decreases in testis weights. Testis weight were only 2% lower than control in the 35 mg/kg-day group and 6% lower than control in the 100/175 mg/kg-day group. This cannot be significant since there mice treated with the lowest 1.5 mg/kg-day also showed a 6% lower testis weight than control. One would certainly expect the rats that showed degeneration to have markedly reduced testis weights, but such an analysis was not performed. Furthermore, the failure of another study to observe any significant decreases in testis weights or histological lesions in a subchronic 13-week study at doses up to 160 and 320 mg/kg-day with the same mouse strain (Cholakakis et al. 1980), further weakens the support for effect indicated in the Lish et al. (1980) study. Although the RDX used by Cholakakis et al. was of larger particle size than that used by Lish, they extended their study to higher doses at which, even though the absorption of the larger particles was not as efficient as with the smaller particles, it was sufficient to induce animal toxicity. Also since there is no evidence of bioaccumulation of RDX, there subchronic study should have been sufficient to show an effect but it did not.

There have been a number of studies in rats that have generally found no significant effect. In a 2-year chronic study, Hart et al. (1976) found no testicular degeneration or weight loss at doses up to 10 mg/kg-day. Similarly Levine found no effect of a dose of 8 mg/kg-day. However, but at 40 mg/kg-day there was a significant decline in testis weight (14%) and a significant increase in the percentage of testes showing germ cell degeneration at 12 months of treatment. Although the effect is significant, the fact that there was 40% mortality by this time may indicate that the testicular damage was secondary to general toxicity. Testis weights at 24 months were not meaningful since all rats of this strain developed Leydig cell hyperplasia/neoplasms by 2 years of age, and the histological results, claiming no germ cell degeneration, are questioned.

There have been 3 studies of testicular effects in 13-week subchronic studies. Two studies used administration by diet as had been done in all the above studies. Cholakis et al. (1980) found no changes in absolute testis weights or histopathological damage to testes at 28 or 40 mg/kg-day. Note that there was some effect of the higher dose on weight gain but no mortality. Similarly Levine et al. (1981a,b, 1990) found no changes in testis weight, degeneration of the seminiferous epithelium or mortality at 30 mg/kg-day. Even though there was 80% mortality at 100 mg/kg-day there were not testicular effects. Finally Couse et al. (2006) using gavage, which generally has greater potency than dietary administration, observed some mortality at doses between 8 and 15 mg/kg-day. Nevertheless there were no significant histological effects and the authors indicated no significant change in absolute testis weights (although by a t-test I found an 8% decrease at 15 mg/kg-day).

Thus, other studies in general fail to support the testicular toxicity observed by Lish et al. (1980). The only other study with a suggestive effect (Levine et al., 1983, results at 1 year), are also associated with animal toxicity leading to appreciable mortality.

3.c.v - **Reproductive system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

Reference dose is calculated from BMDL for a small level testicular degeneration in mice. However, more severe effects on reproduction (pregnancies, fetal survival, offspring survival) are observed in rats and should be given more weight in determination of the Reference Dose for reproductive effects.

Specific comments on presentation of data on reproductive and developmental toxicity

1. In Table 1-9, the relative testes weights should be deleted. Relative testis weight is affected by changes in body weights, which in our experience does not have effects on testis weights of adult animals. Absolute testis weights are a better measure of testicular toxicity of an agent. The relative testis weights just clutter up the table and add little information on the toxicity of RDX.
2. Table 1-9, Page 1-42: In the presentation of the data of Levine et al. (1983), the data on "SDMS" (spontaneous death or moribund sacrifice) rats should be deleted. Their significance is open to question and they aren't given much weight in the discussion.
3. Table 1-9, Page 1-44: The data on incidence of germ degeneration of Levine et al. (1981a,b, 1990) at 12 and 15 mg/kg-day should be deleted. These were observed on dead rats (all rats in these groups died). Incidentally the numbers are reversed: the value for 1/10 was for the 12 mg/kg-day dose and 1/9 was for 15 mg/kg-day.
4. The testis weight data from Cholakis et al. (1980) (Table 1-9, last entry on Page 1-43) on F2 weanlings does not belong in the male reproductive effect section. It is not indicative of direct effect on testis weight and there is no follow-up to determine whether or not adult testis weights will be affected. Rather it belongs in the developmental effects section (Table 1-10).

Additional References

Beattie MC; Adekola L; Papadopoulos V; Chen H; Zirkin BR. (2015). Leydig cell aging and hypogonadism [Review]. *Exp. Gerontol* 68: 87-91.
Zhang, X; Ebata, KT; Robaire B; Nagano M (2008). Aging and male germ line stem cells in mice. *Biol Reprod* 74: 119-124.