

Ethylene Glycol Monomethyl Ether: Litter Two

CAS #109-86-4

Sprague-Dawley rats, at 0.0, 0.01, .03, .1%, drinking water

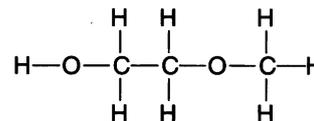
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Reproductive Assessment by Continuous Breeding (RACB) studies were originally designed to be conducted using mice, with the initial justification relating to their size and relative cost (Gulati et al., *Fundam Appl Toxicol* 17:270-279 [1991]). However, the rat is more commonly used for investigative and regulatory studies. There should be no a priori reason why the rat could not be used in a design like the RACB: the two species would appear to share more similarities than differences.

However, one potential problem for the RACB is that when a compound is toxic to reproduction, it often results in sterile matings (or no matings) toward the end of the cohabitation period, which severely reduces the number of offspring available to test in a second generation. A way around this difficulty would be to rear an earlier (i.e., the second) litter for the F_1 evaluation, not the fifth. Thus, this study and the previous rat study were designed to test the possibility that rats could be used in an RACB design, and to compare the rearing of a second versus the fifth litters. The design was modified by extending the length of Task 2 from 14 to 16 weeks to accommodate the slightly longer gestation length in rats. An overall result of this change is that the length of the entire study is reduced in the Litter2 design, by approximately 5 weeks.

Ethylene glycol monomethyl ether (EGME) was used as a reproductive toxicant whose actions are relatively well defined. Task 1, the dose-range-finding portion of the design, was not conducted, as sufficient data were already available to allow dose setting for Task 2. Concentration levels for Task 2 were set at 0.01, 0.03, and 0.1% weight per volume in drinking water. The high dose group consumed approximately

10 to 19% less water than did the controls. These levels of EGME produced consumption estimates of approximately 10, 30, and 90 mg/kg/day. Ten females died during Task 2: four controls, two at the low dose, three in the middle dose, and one in the high dose group. The causes were varied and judged by the study pathologist to be unrelated to treatment.

In the high dose group, only one pair delivered a litter (at the very end of the cohabitation period). While the number of litters per pair was not reduced in the middle dose group, the numbers of live pups per litter was decreased by 42%. Whereas 5% of control pups were stillborn, 26% of pups in the middle dose group were stillborn. In the low dose group, there was a nonsignificant decline in the mean pup number from 12.8 to 11.7, and a 5% increase in pup weight adjusted for litter size.

This study reared the second litter for testing effects in the second generation. In the second generation, no animals were available from the high dose, so F_1 testing was done with controls, low, and middle dose groups. In the middle dose group, postnatal survival was reduced by 33 to 50%, and weight gain was also reduced, so that by weaning, these pups weighed an average of 19% less than their controls.

This change in timing meant that when the crossover mating trial (Task 3) was run with the F_0 rats after the last litter was delivered and removed, the F_0 rats were approximately 4 to 5 weeks older than their counterparts in a standard RACB study. This age difference may have contributed to a slight decline in the number of pups born to control pairs in this study, relative to the "standard" design: 8.1 versus 13.3. In this study, the middle dose was used in the crossover with the controls. There was

no difference in mating or fertility indices or the number of live pups per litter, although the proportion of liveborn was reduced by 11%.

After the delivery and evaluation of the Task 3 pups, all control and middle dose F_0 rats were killed and necropsied. For females, there was no change in body or organ weights. For males, there was a 15% body weight difference at the high dose. In the middle dose group, relative liver weight was reduced by 9%, seminal vesicle weight by 9%, and epididymis weight by 8%. At the high dose, liver, kidney, and seminal vesicle weights were reduced by 13, 10, and 19%, respectively, while testis weight was down 33%, epididymis weight reduced by 40%, and prostate weight reduced by 20%. Sperm end points were affected only at the high dose, with the percentage of motile sperm reduced by 50% and epididymal sperm density reduced by 62%.

In the second generation mating trial, there were no treatment effects on mating or fertility indices, although the number of live pups was reduced by approximately 17% at 0.03%, and the viability of the pups was reduced by approximately 5%. Pup weight was increased by 5% in the low dose group.

After the F_2 pups were delivered and assessed, all rats were killed, and 20 F_1 rats per group were necropsied. Female body weight was reduced by 9% in the middle dose group, but relative organ weights were not affected. Male body weight in the middle dose group was down by 17%. Absolute testis weight was unchanged by up to 0.03% EGME consumption, but this middle dose group suffered reductions in liver, kidney, epididymis and prostate weights of 14, 6, 13, and 28%, respectively. Interestingly, epididymal sperm density was

reduced at both the middle and low doses, by 23 and 17%, respectively.

Thus, EGME was clearly toxic to rat reproduction in this design. The overall study length reduction achieved by this design may be offset by the poorer perfor-

mance of all rats during the crossover, due to their slightly greater age, and by other considerations presented in the publication comparing these two designs. It appears that either design will work with rats, and indeed, rats are used routinely for RACB

studies now, rearing the fifth litter. Two findings of note were the increase in pup weights seen in the low dose in both rat studies, and the reduction in epididymal sperm number observed in this study in the low dose F₁ males.

ETHYLENE GLYCOL MONOMETHYL ETHER: LITTER TWO

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: PB90252313

Chemical: Ethylene Glycol Monomethyl Ether

CAS#: 109-86-4

Mode of exposure: Drinking water

Species/strain: Sprague Dawley rats

F ₀ generation	Dose concentration →	.01%	.03%	.10%
General toxicity		Male, female	Male, female	Male, female
Body weight		—, —	—, —	↓, •
Kidney weight ^a		—, —	—, —	↓, •
Liver weight ^a		—, —	↓, —	↓, •
Mortality		—, —	—, —	—, —
Feed consumption		•, •	•, •	•, •
Water consumption		—, —	—, —	↓, ↓
Clinical signs		—, —	—, —	—, —

Reproductive toxicity				
\bar{x} litters/pair		—	—	•
# live pups/litter; pup wt./litter		—, ↑	↓, —	•, •
Cumulative days to litter		—	↑	•
Absolute testis, epididymis weight ^a		—, —	—, ↓	↓, ↓
Sex accessory gland weight ^a (prostate, seminal vesicle)		↑, —	—, ↓	↓, ↓
Epidid. sperm parameters (#, motility, morphology)		—, —, —	—, —, —	↓, ↓, —
Estrous cycle length		•	•	•

Determination of affected sex (crossover)		Male	Female	Both
Dose level		—	—	—

F ₁ generation	Dose concentration →	.01%	.03%	.10%
General toxicity		Male, female	Male, female	Male, female
Pup growth to weaning		—, —	↓, ↓	•
Mortality		—, —	↑, ↑	•
Adult body weight		—, —	↓, ↓	•, •
Kidney weight ^a		—, —	↓, —	•, •
Liver weight ^a		—, —	↓, —	•, •
Feed consumption		•	•	•
Water consumption		↓, ↓	↓, ↓	•
Clinical signs		—, —	—, —	•

Reproductive toxicity				
Fertility index		—	—	•
# live pups/litter; pup wt./litter		—, ↑	↓, —	•, •
Absolute testis, epididymis weight ^a		—, —	—, ↓	•, •
Sex accessory gland weight ^a (prostate, seminal vesicle)		—, —	↓, —	•, •
Epidid. sperm parameters (#, motility, morphology)		↓, —, —	↓, —, —	•, •, •
Estrous cycle length		•	•	•

Summary information	
Affected sex?	Unclear
Study confounders:	None
F ₁ more sensitive than F ₀ ?	Yes
Postnatal toxicity:	Yes

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change (p<0.05); —, —, no change in males or females. ^aAdjusted for body weight.