

## Reproductive Toxicity Assessment by Continuous Breeding in Sprague-Dawley Rats: A Comparison of Two Study Designs

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Reproductive Toxicity Assessment by Continuous Breeding in Sprague-Dawley Rats: A Comparison of Two Study Designs. GULATI, D. K., HOPE, E., TEAGUE, J. AND CHAPIN, R. E. (1991). *Fundam. Appl. Toxicol.* 17, 270-279. The protocol for Reproductive Assessment by Continuous Breeding (RACB) studies was originally designed to use mice as the test species. However, rats are commonly used for reproductive toxicity research and could be used in the basic RACB design. One of the studies reported below evaluated a standard murine RACB design using rats, which rears the fifth litter to test second generation fertility. The second design tested the logistics and feasibility of rearing the second litter for second generation fertility testing. The standard fifth litter design (L5) was modified slightly for rats by increasing the time allowed for gestation and delivery. Compared to rats rearing their second litter (L2), rats in this L5 design had more litters per pair during continuous breeding and maintained this fertility better over time, as evidenced by producing more pups per litter during the crossover mating segment. Both L2 and L5 rats gave sufficient pups to conduct the second generation fertility evaluation. In addition, the L5 design was easier to conduct and produced pups from gametes exposed to chemical throughout spermatogenesis, making it the preferred design for using rats in Continuous Breeding studies.

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### INTRODUCTION

The National Toxicology Program has developed a protocol for assessing reproductive toxicity in rodents. This protocol, "Reproductive Assessment by Continuous Breeding" (RACB), has been in use for over 9 years with over 70 studies conducted in two laboratories (Morrissey *et al.*, 1989). The RACB design includes a 14-day range-finding segment known as Task 1, a 14-week continuous breeding phase (Task 2), an optional crossover mating to identify the affected sex (Task 3), and F<sub>1</sub> reproductive assessment, called Task 4 (Fig. 1 [see also Morrissey *et al.*, 1989, for a more complete description]). One strength of the RACB protocol lies in the design of Tasks 2 and 3. During continuous cohabitation in

Task 2, the Swiss mouse can produce up to five litters of pups. The early litters of Task 2 are killed immediately after delivery and data collection, thereby encouraging postpartum estrus and precluding delayed implantation. Cohabiting pairs are separated after 14 weeks and the last (fifth) litter is retained, monitored through nursing, and used for fertility testing at sexual maturity. When all final litters are weaned, the F<sub>0</sub> control mice can be cross-mated with a treatment group showing positive reproductive effects as a way of identifying the affected sex. Since the animals begin Task 2 cohabitation 1 week after the start of treatment, more data are collected for the same time period than in the standard two generation reproduction design, where cohabitation does not begin until the completion of one

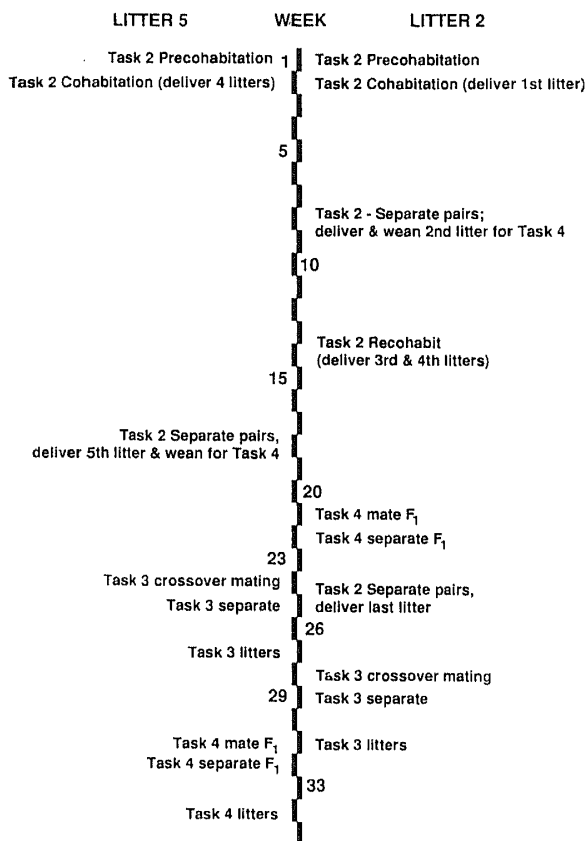


FIG. 1. Time line showing significant events in the rat Litter 5 design (left) and Litter 2 design (right). The alternating line between the two represents study weeks, with the first week at the top. Animals are necropsied after delivering the final litter (bottom).

spermatogenic cycle. Since litters are produced over 14 weeks, the RACB design can distinguish cumulative toxic effects or indicate possible sensitive phases of spermatogenesis. Task 2 data provide a preliminary estimate of reproductive toxicity; if results are negative, Task 3 is not conducted and Task 4 is conducted with control and high dose animals only. If Task 2 shows toxic effects, Task 3 is performed and Task 4 uses control and all three dose groups.

To date, the RACB studies have been conducted with mice only, and recent efforts have concentrated on developing the study design for use with rats. Considerations in designing the rat studies included: suitability of the original design to rats, thereby maintaining consistency with the mouse protocol; logistics and cost of routinely performing rat continuous

breeding studies; and continued fertility and productivity of the continuous breeding pairs. This last consideration was crucial to the success of this study design, which assumes (1) that most control pairs would be capable of producing five litters in rapid succession; (2) that enough final (fifth) litters would be available to test the F<sub>1</sub> without resorting to sibling matings; and (3) that parental fecundity would remain high enough to test for the affected sex in Task 3. While these assumptions are valid when using Swiss CD-1 mice, they had been evaluated only once in rats. In a pilot study with F344 rats, it was found that only 5 of 40 control pairs produced all five litters, and only half the pairs produced four litters (NTP, 1986). The present studies used a more fertile strain, the Sprague-Dawley, and attempted (1) to determine the suitability of the original RACB design for Sprague-Dawley rats and (2) to test a modified design which would accommodate any decline in fertility by still producing sufficient animals to run Task 4. The modified design rears animals from the second litter to use in the F<sub>1</sub> test (Task 4).

To evaluate the rat protocols, ethylene glycol monomethyl ether (EGME) was used as a positive control. EGME is a known teratogen and male reproductive toxicant in several species (Nagano *et al.*, 1979; Hardin *et al.*, 1982; Nelson *et al.*, 1982; Foster *et al.*, 1983; Mebus and Welsch, 1989). Furthermore, it has been thoroughly tested using the RACB protocol in mice (NTP 1985, 1988a,b,c). It was felt that these data would allow dose levels to be set without Task 1 data and studies to be started with Task 2.

## MATERIALS AND METHODS

Detailed accounts of the RACB study design have been published elsewhere, including animal housing and quarantine procedure, randomization of animals into treatment groups, and frequency and methods of dosage analysis and data collection procedures (Heindel *et al.*, 1989). Only major points are included here.

*Animals.* VAF Crl:CD BR outbred Sprague-Dawley rats were obtained from Charles River Breeding Labs (Portage, MI). Rats were quarantined for 2 weeks upon arrival and

were 12 weeks old at the start of chemical exposure (13 weeks old at the beginning of Task 2 continuous breeding). Rats received feed (NIH-07 pellet diet, Zeigler Bros., Gardner, PA) and deionized, filtered water *ad libitum*. Forty continuous breeding pairs were assigned to the control group for Task 2 continuous cohabitation, while 20 pairs were assigned to each of three treatment groups. Standard Task 3 protocol was followed, where control rats were reassigned so that half of the control males mated with females of the selected treatment group, half of the control females mated with treated males, and the remaining control animals mated with each other. For Task 4, 20 mating pairs were selected from the pool of F<sub>1</sub> rats in each treatment group, avoiding sibling matings.

**Study design.** The protocol designated "Litter 5" or "L5" followed the standard RACB pattern except that the Task 2 continuous breeding period was increased to 18 weeks to allow for the longer gestation period of rats (22 days vs 19 days in Swiss CD-1 mice). One holding period occurred in Task 2, just before the expected delivery of the fifth litter; pups from this last litter were reared for Task 4. The Tasks 3 and 4 holding periods increased slightly, again to accommodate the increased gestation length and the later sexual maturation of the rats (approximately 84 days vs 74 days for Swiss CD-1 mice) increased the time between F<sub>1</sub> weaning and mating.

The other study design ("Litter 2" or "L2") was an attempt to compensate for a possible decline in Task 2 fertility/fecundity which would preclude testing F<sub>1</sub> fertility in Task 4. In the L2 design, the Task 2 continuous cohabiting pairs were separated just before they were expected to deliver their second litter. These litters were reared, weaned, and retained for Task 4 matings. After these litters were weaned, the Task 2 F<sub>0</sub> rats were re-paired long enough to produce three more litters, which were not retained. By this design, even if F<sub>0</sub> fertility had declined to naught by the fifth litter, sufficient F<sub>1</sub> animals would be present to test second generation fertility. Figure 1 shows the time sequence and progression of the two protocols. Both protocols followed the standard procedure for Task 3 (1 week of cohabitation or until evidence of mating, whichever is less) and Task 4 (1 week of cohabitation regardless of evidence of mating). However, because of the differences in conducting Task 2, Tasks 3 and 4 occurred at different timepoints in the two studies, and the F<sub>0</sub> rats in the L2 design were older at the start of Task 3 (Fig. 1).

**Test chemical and dose formulations.** EGME was obtained from General Drug and Chemical Corp. The EGME was >99% pure, as determined by GC and HPLC analysis. Chemical was added to drinking water on a weight/volume basis. Fresh dosing formulations were made every 3 weeks or less. Representative formulations were analyzed and found to be within  $\pm 10\%$  of theoretical values. Rats received treatment continuously from the beginning of Task 2 (from weaning for F<sub>1</sub> rats) until necropsy at the end of Task 3 or 4. Drinking water was changed at least twice each week.

Task 2 dose levels of 0.01, 0.03, and 0.10% EGME were chosen prospectively for the two studies. The L2 study was initiated before the L5 study began and it was apparent very early that the 0.10% dose group would be essentially infertile and that the 0.03% group would also show some positive effects. However, it was unclear whether a cumulative effect would appear in the 0.01% group. In order to achieve a "no observed effect level" for EGME in rats, concentrations used in the subsequent L5 study were 0.006, 0.012, and 0.024%. The Task 4 segments were conducted with all doses (except for the 0.10% group, since only one litter was born in this group). Task 3, the crossover mating, used the high dose in L5 (0.024%) and the mid dose in L2 (0.03%).

**Data collection.** The reproductive endpoints in Tasks 2, 3, and 4 included fertility (defined as producing at least one pup, dead or alive), number of live pups per litter, proportion of live pups per litter, and mean live pup weight (sexes weighed separately). In addition, Tasks 3 and 4 endpoints included mating index (number of females exhibiting vaginal plug or sperm). Other data collected in Tasks 2, 3, and 4 included mortality, and clinical signs of toxicity, body weight, drinking water consumption, and dam weight at each delivery (see Heindel *et al.*, 1989 for further details). For the litters retained during the Task 2 holding period (first holding period for L2 rats or the only holding period in L5), pup body weights and survival to Postnatal Day 21 were monitored. Rats were necropsied at the end of Tasks 3 and 4: organs were weighed; epididymal sperm was evaluated for density, motility, and sperm head abnormalities; and selected tissues and dose groups were evaluated microscopically. Here, however, only the reproductive endpoints are discussed, as detailed results for each study (body weights, individual litter data, etc.) are available elsewhere (NTP, 1989a,b).

**Statistical analyses.** Within the L2 and L5 studies, results were compared to those of the specific study control group. In Task 3, where dose groups do not represent increasing dose levels, a  $\chi^2$  test for homogeneity (Snedecor and Cochran, 1967) was used to test for an overall difference in fertility among groups. Pairwise comparisons between the control and treatment groups were made with Fisher's exact test (Conover, 1971). For Task 2 the number of litters and the number of live pups per litter were computed on a per-fertile-pair basis and then treatment group means determined. For the multiple litters of Task 2, the proportion of live pups was defined as the number of pups born alive divided by the total number of pups produced by each pair. Dose group means for these parameters were tested for overall differences using the Kruskal-Wallis test (Kruskal and Wallis, 1952) and for ordered differences using Jonckheere's test (Jonckheere, 1954). To remove the potential effect of the number of pups per litter on the average pup weight, an analysis of covariance was used (Neter and Wasserman, 1974) with the covariate being litter size, live and dead pups combined. Least squares estimates of dose group means, adjusted for litter size,

were computed and tested for overall equality using an *F* test. The covariate analyses of body weight were performed on sexes separately as well as combined.

The control data from the L2 and L5 studies were compared and analyzed *post hoc*. Task 2 data were analyzed in two ways, comparing data for combined litters as described above and comparing data for first, second, third, fourth, and fifth litters separately. Effects on overall fertility were tested with Fisher's exact test (Conover, 1971). Mean litters per pair, live pups per litter, and proportion of live births (litters combined) were analyzed using the Wilcoxon test. Adjusted live pup weight was analyzed using the Student *t* test (Neter and Wasserman, 1974). Mean litter size over time was analyzed using a repeated measures analysis of variance (Neter and Wasserman, 1974), followed by pairwise *t* tests comparing studies by litter. The Bonferroni method was used to establish the significance level for the litter by litter comparisons (Neter and Wasserman, 1974).

Tasks 3 and 4 fertility and litter parameters were analyzed in the same manner as for the Task 2 combined litter analyses; effects on the Tasks 3 and 4 mating indices were tested using Fisher's Exact test.

## RESULTS

Although these studies were performed primarily to examine the reproductive performance of controls in two different designs, it was important to show that a RACB design using rats would identify a reproductive toxicant. Figure 2 summarizes some of the reproductive toxicity seen with EGME exposure. Figure 2A shows the effects of EGME on the mean number of live pups per litter from both studies. For each dose group in each study, these data are summed over all litters. As the concentration of EGME increases, the mean number of live pups per litter decreases. Figure 2B shows the proportion of pups born alive for both studies; as EGME concentration increases, the proportion of live births decreases. In the 0.1% group in L2, there was only 1 litter delivered by 1 pair of a possible 100 litters (20 pairs  $\times$  5 possible litters). In that 1 litter, 89% of the pups were born alive. In addition, exposure to EGME caused a dose-related increase in days to delivery in both studies (Table 1). However, data on days to delivery are shown for only one study (L5) because the L5 study had more pairs delivering pups, thus providing increased confidence in the data.

Table 2 compares the reproductive performance of the control pairs in the L2 and L5 studies during Task 2 (combined litter data per pair). No significant differences occurred in fertility, live pups per litter, or proportion of live births. The mean number of litters per pair was significantly lower in L2 compared to in L5, while adjusted live pup weight was significantly increased. When the Task 3 control  $\times$  control matings are compared (Table 3), the L2 study shows a significant decrease in the proportion of pairs mating compared to L5, and a significant increase in the proportion of live births, but no difference in the number of fertile pairs, number of live pups per litter, or adjusted live pup weight.

Figures 3 and 4 show Task 2 data on a per litter basis, with Task 3 data included for comparison. There were no statistically significant differences between the two designs in the proportion of pairs delivering each litter, although the values for the L2 rats were less than those of the L5 pairs at each litter except the first (Fig. 3).

Figure 4 compares control litter size (live and dead pups per litter) over time for both studies. In L5, litter size declined gradually with time, but rose again to first-litter values during Task 3. In contrast, litter size in L2 increased slightly at the third litter (when  $F_0$  pairs were recohobated) and then declined slightly during Task 2 and sharply at Task 3. By repeated measures analysis of variance, a significant litter size effect occurred in the L2 study, reflecting the overall decrease in litter size with time. A significant study  $\times$  litter interaction indicates that the pattern of litter size decrease is dissimilar in the two studies. However, there was no significant difference between the two studies in the litter size during Task 2. During Task 3, the L5 pairs produced significantly more pups than did the L2 controls; 11/18 pairs in L2 produced no pups at all during Task 3.

Both protocols provided sufficient litters to conduct Task 4. Thirty-six of forty (90%) L2 control pairs produced litters during their first holding period (Days 46-87), with a range of

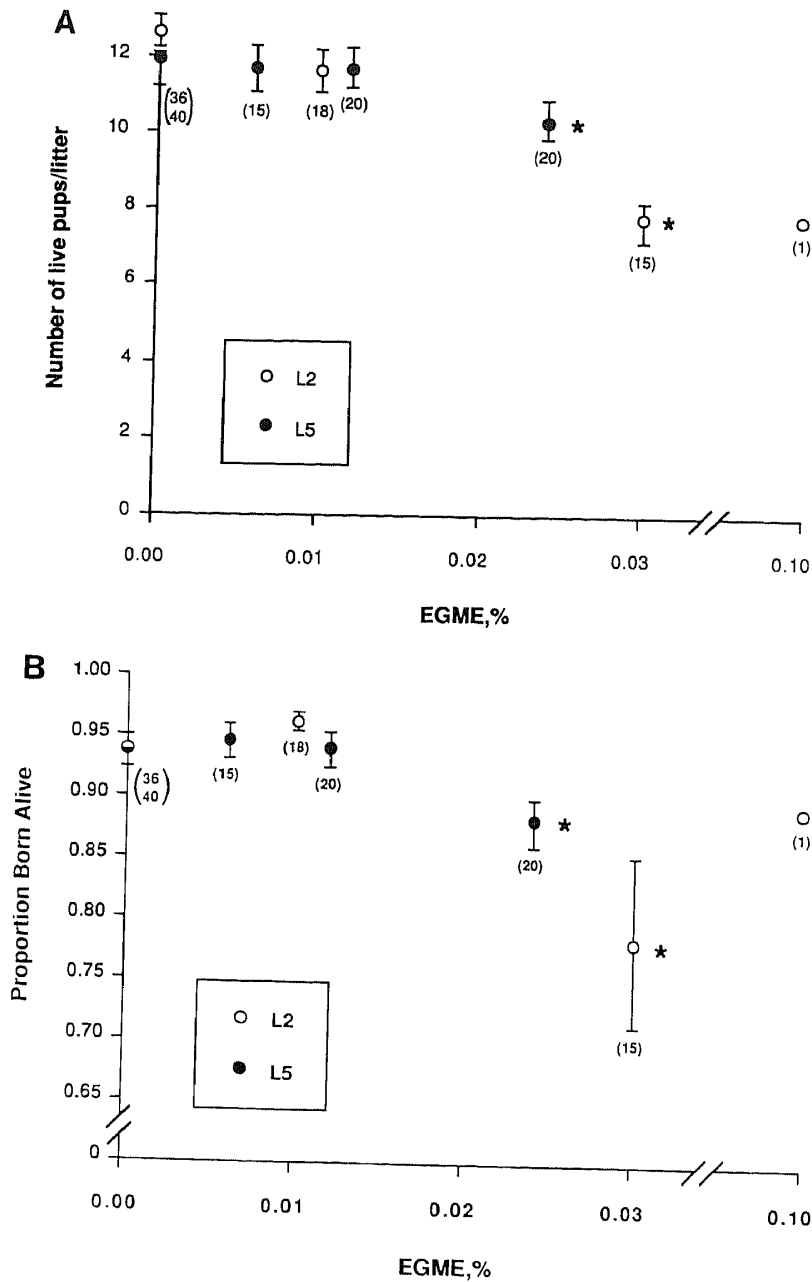


FIG. 2. (A) The effects of EGME on the mean ( $\pm$ SEM) number of live pups per litter in the L2 and L5 studies. Numbers in parentheses indicate the number of pairs in that dose group that delivered at least 1 litter. Only one pair in the 0.10% EGME group had a litter. \*Different from control,  $p < 0.05$ . (B) The effects of EGME on the mean ( $\pm$ SEM) proportion of pups born alive. Both control groups had the same value;  $n$  for L2 is 36,  $n$  for L5 is 40. In the one litter delivered in the 0.10% group, one of nine pups was delivered dead. \*Different from control,  $p < 0.05$ .

2 to 16 pups per litter. During the L5 holding period (Days 112–154), 28/40 (70%) control pairs produced litters, with a range of 3 to 16 pups per litter. (The number of litters produced during the holding period is not necessarily the number of second or fifth litters

produced; as an example, pairs could deliver their fourth litter during the holding period.) While the number of litters and the average litter size were both lower in the L5 study (9.7 pups per litter in L5 vs 12.4 for L2), the pool of nonsibling rats was still more than suffi-

TABLE 1  
CUMULATIVE DAYS TO LITTER, L5 STUDY (TASK 2)

Treatment group	Litter 1	Litter 2	Litter 3	Litter 4	Litter 5
Control	24.5 ± 0.4 (40)	48.7 ± 1.4 (40)	72.9 ± 1.5 (38)	97.3 ± 1.6 (35)	116.8 ± 0.9 (24)
0.006%	24.9 ± 0.5 (20)	47.8 ± 0.5 (20)	70.7 ± 0.6 (19)	94.7 ± 1.2 (19)	119.2 ± 1.3 (17)
0.012%	26.6 ± 1.5 (20)	49.5 ± 1.5 (20)	73.0 ± 1.5 (20)	100.4 ± 2.6 (20)	121.7 ± 1.7 (16)*
0.024%	25.6 ± 0.5 (20)*	51.8 ± 1.2 (20)*	77.1 ± 2.0 (20)*	100.4 ± 2.0 (19)*	121.5 ± 1.4 (15)*

Note. Mean ± SE; number of animals providing the data indicated in parentheses. Note that the high dose group took significantly longer to deliver each litter, starting with the first litter. The middle dose group took longer to deliver the last litter.

\* Different from controls at this litter,  $p < 0.05$ .

cient to select 20 nonsibling pairs for Task 4 mating.

Task 4 control results from the L2 and L5 studies did not differ in terms of pup survival to weaning, weight gain, mating performance, fertility, or litter parameters (data not shown).

## DISCUSSION

The statistical sensitivity of this design would be optimal if the test species was capable of full fecundity even in aged animals. This would allow five litters to be produced during Task 2, so that second generation animals would be produced from sperm exposed to test chemical throughout all stages of spermatogenesis. The ideal species would also permit full fertility in the Task 3 crossover mating 6 weeks after the end of Task 2. The Swiss mouse has been the species used in Continuous Breeding studies because of its small size and high fecundity, but has shown lower fertility in Task 3 (see Morrissey *et al.*, 1989). Rats are more commonly used for mechanistic studies and testing to meet regulatory needs. The present studies were undertaken to see how well the Sprague-Dawley rat could be used in this design (L5) and to test an alternative design (L2) that would increase the chances of having a full complement of second generation animals to test. The logistics of each design also merit consideration. The following will

show that, by most of these criteria, we believe the L5 design is generally superior to L2.

When comparing Task 2 control results on a litter by litter basis, it is evident that fertility declines in both studies. However, this decline is more marked with L2 rats (Fig. 3). The decline in fertility may be due in part to animal age. Because of the necessarily long interval for delivery and weaning of the second litter, the F<sub>0</sub> pairs in the L2 study are approximately 6 weeks older than the L5 pairs at the delivery of the third, fourth, and fifth litters. The L5 pairs actually delivered their last litter at the time L2 pairs delivered their third litters, Study Week 17 (rats were approximately 28 weeks of age). Even at the end of Task 2, the L2 rats are 3 weeks older, because the L2 design requires an additional holding period of up to 3 weeks to allow for the delivery and termination of the final, fifth litters.

If age were the only factor affecting fertility, however, the L5 Task 3 fertility should be similar to L2 fifth litter fertility when rats in both cases are 35 weeks of age. Instead, L5 rats were more fertile than L2 rats at this age (compare bars in Figs. 3 and 4). While the defined causes of this are unclear, we might speculate that the greater fertility decline in the L2 design may be due to cycles of sexual inactivity in the males. In production colonies, the Sprague-Dawley breeder males routinely are kept until approximately 40 weeks of age and continuously cohabited with several fe-

TABLE 2  
REPRODUCTIVE PERFORMANCE OF CONTROL PAIRS DURING CONTINUOUS BREEDING (TASK 2)

	No. fertile <sup>a</sup> /No. cohoused (%)	Litters per pair	Live pups per litter	Proportion live births	Live pup weight <sup>b</sup> (g)
Litter 5	40/40 (100)	4.4 ± 0.1 <sup>c</sup>	11.9 ± 0.3	0.94 ± 0.01	6.09 ± 0.05
Litter 2	39/39 (100)	3.9 ± 0.2*	12.6 ± 0.3	0.94 ± 0.01	6.36 ± 0.07*

*Note.* Control animals in the L2 study produced, on average, fewer litters per pair, although the weight of the live pups in those litters was greater compared to that of the L5 control rats.

<sup>a</sup> A pair was considered fertile if they delivered at least one pup, dead or alive.

<sup>b</sup> Least squares estimate of mean pup weight adjusted by analysis of covariance.

<sup>c</sup> Data are presented as mean ± SEM.

\* Different from Litter 5,  $p < 0.05$ .

males which are replaced as they become pregnant (Charles River Technical Bulletin, 1982; personal communication, Charles River Breeding Laboratories, 1988). Hence the potential for sexual activity is almost uninterrupted. In contrast, males in the RACB studies might have receptive partners only every 3 weeks (if fertilization occurs), and during the holding periods (6 weeks for L5, 6 and 3 weeks for L2) the males were entirely separated from their mates. However, fertility is also related to females, and it is likely that their ability to conceive and deliver young declines with age. Only further work will clarify this question.

In terms of Task 2 data generated, the L5 protocol appears superior to the L2 protocol. More of the L5 pairs had fourth and fifth litters, although the size of the fifth litter was not

significantly different from those of L2. Comparing Task 3 results, the L5 design appears superior, with a much higher mating index and number of pups per litter. However, both studies showed intermediate to poor control fertility (proportion of pairs delivering a litter) in Task 3, making it less likely that one could distinguish marginal effects in the treated × control matings. Declining fertility at Task 3 is an occasional problem in RACB studies conducted in mice. However, a modified design is being tested wherein naive adults are introduced to mate with treated or control partners. Preliminary data from this study suggest that the fertility decline is reduced.

Despite the declining fertility, both protocols produced sufficient young for a Task 4 second generation fertility evaluation. This has

TABLE 3  
REPRODUCTIVE PERFORMANCE OF CONTROL PAIRS DURING CROSSOVER MATING TRIAL (TASK 3)

	No. mated/No. cohoused <sup>a</sup> (%)	No. fertile/No. mated (%)	Live pups per litter	Proportion live births	Live pup weight <sup>b</sup> (g)
Litter 5	19/19 (100)	12/19 (63)	13.3 ± 0.7 <sup>c</sup>	0.90 ± 0.03	6.55 ± 0.17
Litter 2	13/18* (72)	7/13 (54)	8.1 ± 1.9	1.00 ± 0.00*	6.71 ± 0.19

<sup>a</sup> A pair was considered mated if the female showed a copulatory plug or sperm in the vagina.

<sup>b</sup> Least squares estimate of mean pup weight adjusted by analysis of covariance.

<sup>c</sup> Data are presented as mean ± SEM.

\* Different from Litter 5,  $p < 0.05$ .

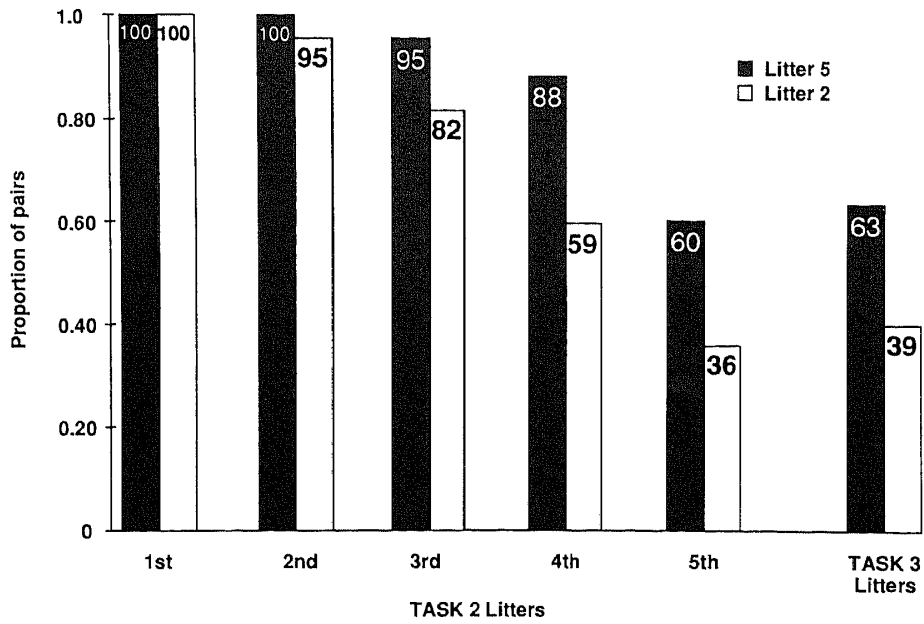


FIG. 3. Proportion of control pairs delivering each litter. Although compared to L5 controls, fewer controls in the L2 study delivered a second, third, fourth, or fifth litter, or a litter during the Task 3 crossover, these differences were not significant.

also been the case in several subsequent rat studies using the L5 design (not shown).

Additionally, the final litter in the L5 design yields pups produced by sperm exposed to test

chemical throughout the entire process of spermatogenesis. In contrast, in the L2 design the final litter of pups, produced from these continuously exposed gametes, is not reared

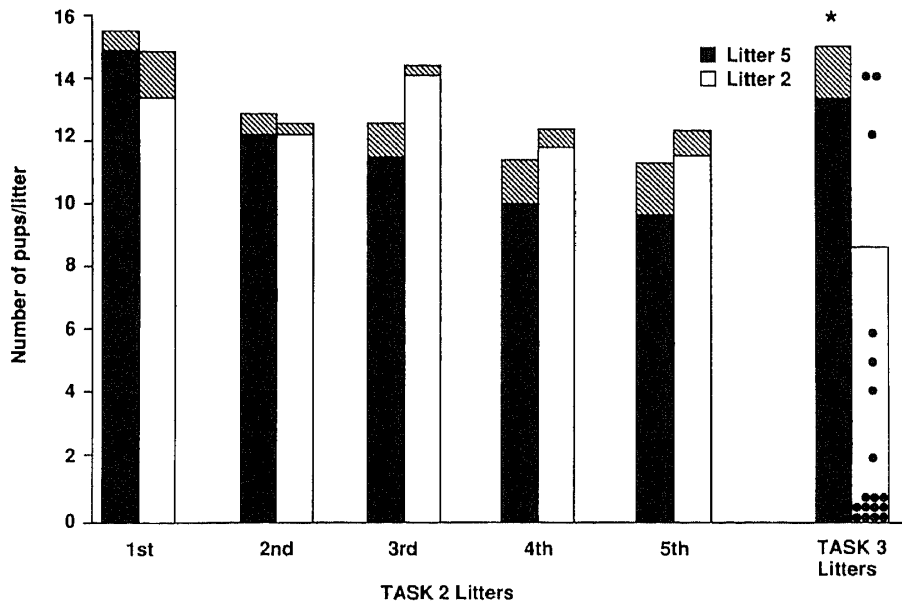


FIG. 4. Mean litter size and number of live and dead pups per litter. Crosshatched areas in each bar represent the mean number of dead pups per litter. While there were no differences between the different designs during Task 2, the controls in the L2 study produced significantly fewer pups per litter during Task 3; dots in the last column represent individual litter data. Eleven of the L2 pairs had no live pups in Task 3 (dots across the bottom of the column). \*Different from control at  $p < 0.05$ .

to maturity, but is terminated shortly after birth. Thus, any paternally mediated effects that might be manifest during maturation would be missed.

A comparison of study length seems to favor the L2 design, since it requires 31 weeks vs 34 for the L5 design. However, the time savings may be outweighed by other logistical considerations. In a standard RACB study, the decisions of whether or not to conduct Task 3, and which dose groups to wean for Task 4 evaluation, depend on the Task 2 litter data. In the standard L5 design, if the data up to the Task 2 holding period are negative, Task 3 is not conducted, and only control and high dose pups are retained for Task 4. Thus, while the labor involved in Task 2 is constant for both positive and negative studies, subsequent labor needs vary greatly between positive and negative studies. The labor, materials, and animal room space needed for a Task 4 with all dose levels are equal to those of a Task 2, while Task 3 requires about two-thirds as much effort as a Task 2. In the L2 design, Task 4 cohabitation begins before Task 2 data collection is complete; therefore, Task 4 must always be conducted as if for a positive chemical, whether or not the test article proves toxic. Conducting both Tasks 2 and 4 simultaneously can complicate resource allocation and involve a waste of labor and material if the test chemical does not prove positive in Task 2.

These studies used lower concentrations of EGME than were used for previous mouse studies (NTP, 1985). In the study with Swiss CD-1 mice, 0.1% was the low dose, which produced slight elevations in days to delivery and a statistically significant 18% decrease in number of live pups per litter, while not changing the proportion born alive. In the present rat L2 study using 0.1% EGME as the top dose, there was just one litter of pups delivered, while the other pairs in the 0.1% group were completely infertile. In the L5 rat study, using 0.024% EGME as the top dose, there was a significant 14% decrease in live pup number, slightly less than the 18% change seen

with the mice. This comparison suggests that the Sprague-Dawley rat is approximately three times as sensitive as is the Swiss mouse to the effects of EGME, at least in this chronic dosing regimen. Finally, we cannot directly compare the relative sensitivity of the two rat designs because different doses were used. However, both designs do appear capable of detecting EGME toxicity.

Some of the differences between the control data from these L2 and L5 studies may be spurious and of little consequence (for example, the pup weight differences in Table 2 or the number mated/number cohabited differences in Table 3), and a replicate set of studies would be required to identify those differences that remain meaningful. Nevertheless, the current data clearly show that the existing RACB protocol (L5) is adaptable for use in Sprague-Dawley rats with only minor changes. Although the modified (L2) protocol provides a larger pool of candidate animals for F<sub>1</sub> testing, it is otherwise inferior in data generated, gamete exposure, and practical operation. The L5 design is now in use by the NTP for Continuous Breeding studies using rats.

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