Feed Restriction in Swiss CD-1 Mice

No CAS #
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A common goal in dose setting for toxicology studies is that by the end of the study the animals in the high dose groups should weigh ±10% less than controls (Chapin et al., Fundam Appl Toxicol 20:15–22 [1993]). This can be due to direct compound toxicity but is also often due to reduced feed consumption. This confounds the interpretation of the resulting data since reproductive processes are known to be affected by the nutritional status of the animal. Separating compound-induced changes from body weight-induced changes in reproductive end points in such cases is effectively impossible.

These three studies were designed specifically to address the impact of restricted feed intake alone on reproductive performance. The intent was to mimic the situation when animals on study eat poorly and have reduced weight gain. In this situation, there is reduced intake of both calories and vitamins and minerals. Thus, diets were not supplemented with vitamins or minerals.

It seemed intuitively obvious that, due to the enormous nutritional demands placed on the pregnant animal, feed restriction (FR) would impair pregnancy outcome in some or all of the restricted females. Less obvious was the amount of FR required to affect the common necropsy parameters measured at the end of 90-day subchronic studies, male fertility, or the early stages of pregnancy (before nutritional requirements rise sharply). Thus, these studies focused on those more common measures of reproductive health taken at necropsy.

The levels of FR were chosen to maintain the animals' body weight at 90, 80, and 70% of concurrent control body weight (CBW). One mouse study was conducted at each of two different laboratories; one rat study was conducted.

For this study, males and females were were housed separately, and fed daily amounts of feed expected to maintain their body weights at target values. Males were tested for fertility at study weeks 8 and 15 by cohabiting with two unrestricted females/male. After the last mating, males were killed and necropsied. FR females were evaluated for estrous cycle prior to, in the middle of, and at the end of the 16-week FR period. After the last "smearing," females were each cohabited with a non-FR male and killed on gestational day 14 to assess ovulation and implantation. Necropsy end points included organ weights and sperm measures.

This first study used Swiss CD-1 mice. After a 5-week reduction period, mice were given an amount of feed that would maintain them at the target weight. This FR paradigm resulted in body weights that were within 7% of target. Blackening of the tail tip was a common clinical sign that was more prevalent and severe with increasing degree of FR. In the first male mating trial (study week 8), the number of litters per male (maximum = 2) was reduced in all FR groups, though the number of pups per litter and their weight was unchanged. The second male mating trial (week 15) showed that the 70% CBW group had half the number of litters per male, and the litters were approximately 30% smaller.

FR females were mated at week 15 to unrestricted males and killed on gestational day 14. While the 90% CBW group was not significantly affected, the number of live and total implants in the 80 and 70% CBW females were reduced by approximately 50%; postimplantation loss was not significantly increased. Vaginal cyclicity was assessed prior to, in the middle of, and at the end of FR: cycles were lengthened only in the 70% CBW group in the middle and end of FR, and the number of acyclic mice was increased in this group at these times.

At necropsy, body weight in the FR females was 87, 76, and 68% of controls; relative kidney weights were increased in the 80 and 70% CBW groups by approximately 30%.

In males at necropsy, body weight was 88, 82, and 72% of control. Absolute testis weight was reduced only at the "high dose," by 12%. Other organ weights were more affected by FR: absolute liver, kidney, and epididymis weights were reduced even at the 90% CBW level. Generally, organ tended to be reduced less than body weight, so that their adjusted weights were increased at the middle and high "doses" (80 and 70% CBW). Epididymal sperm density (numbers per milligram cauda) were reduced only at 70% CBW (by 17%), while the proportion of abnormal sperm was approximately doubled in the 80 and 70% groups. Total spermatids/testis at 70% CBW were reduced by approximately 20%, which is a greater reduction than the 12% reduction in absolute testis weight, indicating impaired spermatogenesis. This was confirmed histologically as foci of impaired and disorganized spermatogenesis.

In summary, although 10% feed restriction was sufficient to reduce the numbers of litters/male during the weight-reduction period, other end points were not affected until 80% CBW, at which point both male (sperm morphology) and female (implant number) indices were affected. The effects became more severe at 70% CBW.

Summary: NTP Reproductive Assessment by Continuous Breeding Study.

NTIS#: **90262551**
Chemical: **Feed Restriction**
CAS#: **NA**
Mode of exposure: **NA**
Species/strain: **Swiss CD-1 mice**

F_0 generation Restriction levels→	90% BWT	80% BWT	70% BWT
General toxicity	Male, female	Male, female	Male, female
Body weight	↓ , ↓	↓ , ↓	↓ , ↓
Kidney weight[a]	— , —	↑ , ↑	↑ , ↑
Liver weight[a]	— , —	↑ , —	↑ , —
Mortality	— , —	— , —	— , —
Clinical signs	↑ , ↑	↑ , ↑	↑ , ↑
Reproductive toxicity, male mating trial 1			
x̄ litters/male	↓	↓	↓
# live pups/litter; pup wt./litter	— , —	— , —	— , —
Reproductive toxicity, male mating trial 2			
x̄ litters/male	—	—	↓
# live pups/litter; pup wt./litter	— , —	— , —	↓ , —
Absolute testis, epididymis weight[a]	— , ↑	— , ↑	↓ , —
Sex accessory gland weight[a] (prostate, seminal vesicle)	— , —	— , —	— , —
Epidid. sperm parameters (#, motility, morphology)	— , — , —	— , — , ↑	↓ , — , ↑
Reproductive toxicity, female mating trial			
Live implants per animal	—	↓	↓
Total implants per animal	—	↓	↓
Post implantation loss	—	—	—
Estrous cycle length, prior to diet restriction	—	—	—
Estrous cycle length, during diet restriction	—	—	↑
Estrous cycle length, after diet restriction	—	—	↑

Summary information	
Affected sex?	Both
Study confounders:	None

Legend: —, no change; •, no observation; ↑ or ↓, statistically significant change ($p<0.05$); — , —, no change in males or females. [a]Adjusted for body weight.