# Data Evaluation Record

**FENVALERATE**
**PC Code 109301**

**EPA Contract No. EP10H001452**
**Task Assignment No. 1-33-2010**

**Study Type: Non-Guideline**

**Citation:** Zhang, H. et al. (2009) Lactational fenvalerate exposure permanently impairs testicular development and spermatogenesis in mice. *Toxicology Letters* 191: 47-56.

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# Data Evaluation Record

**TXR No:** 0055634

**STUDY TYPE:** Non-guideline

**DP BARCODE:** D379859

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**CAS NO.:** 51630-58-1

**MRID NO.:** 48315001

**TEST MATERIAL:** Fenvalerate (purity not reported)

**CITATION:** Zhang, H.; Wang, H.; Ji, Y.; Ning, H.; Yu, T.; Zhang, C.; Zhang, Y.; Zhao, X.; Wang, Q.; Liu, P.; Meng, X.; and Xu, D. (2009) Lactational fenvalerate exposure permanently impairs testicular development and spermatogenesis in mice. *Toxicology Letters* 191: 47-56.

**SPONSOR:** This journal article was submitted as "Other Scientifically Relevant Information" (OSRI) in response to the Agency's Test Order for Tier 1 screening assay for the Endocrine Disruptor Screening Program.

**EXECUTIVE SUMMARY:** The purpose of the study (MRID No. 48315001) was to investigate the effects of maternal fenvalerate exposure during lactation on testicular development and spermatogenesis in male mice offspring. Sperm analysis, histology and immunohistochemistry were performed on the epididymides and testes of the male offspring. Additionally, testosterone synthesis, and the expression of steroidogenic acute regulatory (StAR) protein and testosterone synthetic enzymes (cytochrome P450 cholesterol side-chain cleavage enzyme [P450scc], cytochrome P450 17α-hydroxysteroid dehydrogenase [$P450_{17\alpha}$], and 17β-hydroxysteroid dehydrogenase [17β-HSD]) in the serum and testes were evaluated.

Female ICR mice (8-10 weeks old; weighing 24-26 g) obtained from Beijing Vital River were co-housed overnight with males of the same strain (4 females/2 males). The following morning the females were checked for signs of mating. The day that the presence of a vaginal plug was observed was designated gestation day 0 (GD 0). Within 24 h following delivery, the litters were

culled to 10 pups/litter. Fenvalerate (obtained from Sigma Chemical Co.; purity not reported) prepared in corn oil was administered daily via gavage to maternal mice at a concentration of 60 mg/kg BW during lactation, postnatal day (PND) 0 through 21. Additionally, a control group of maternal mice was similarly treated with corn oil alone during lactation.

At weaning (PND 21), twelve male pups from six litters in each group were sacrificed (method not reported). Serum was collected for measurement of testosterone. The testes were removed and weighed. The left testis was stored at -80°C for subsequent measurement of testosterone, real-time PCR, and immunoblotting. The right testis was immersed in modified Davidson's fluid for 12 h for histology and apoptosis analysis.

To investigate the effects of maternal fenvalerate exposure during lactation on the fertility of male offspring, adult males (PND 80; 12/group) from treated mothers were housed with untreated females. Additionally on PND 80, twelve male pups from six litters in each group were sacrificed and serum was collected for measurement of testosterone. The epididymides were removed for analysis of sperm quality, and the testes were removed and weighed. The testes were treated as described above for weanling offspring.

For RT-PCR, StAR, $P450_{17\alpha}$, 17β-HSD and P450scc, mRNA were normalized to the GAPDH mRNA level in the same samples. The StAR, $P450_{17\alpha}$, 17β-HSD and P450scc mRNA level of the control was assigned as 100%. For immunoblotting studies, StAR, $P450_{17\alpha}$, 17β-HSD and P450scc were normalized to the β-actin level in the same samples. The densitometry unit of the control was assigned as 1. All quantified data were expressed as means ± SEM at each point. Student's *t*-test was used to determine differences between the treated animals and controls; significance was determined as $p<0.05$.

No signs of maternal toxicity or effects on body weight gain during lactation were observed in the treated dams. In addition, maternal treatment with fenvalerate had no effect on the body weights of the pups at PND 21. Absolute and relative testes weights in males from dams treated with the test material during lactation were significantly decreased ($p<0.01$) by 7-13% compared to controls at PND 21. Morphological analysis of the testes from the treatment group on PND 21 showed 4/6 offspring males had testes with more than one large vacuole within the seminiferous tubules per testis cross-section. Moreover, 1/6 treatment group male pups had testes that contained more than one seminiferous tubule with complete spermatogenic failure per testis cross-section. Leydig cells in the testes at weaning (PND 21) were determined by immunostaining for 3β-HSD. The number of Leydig cells in the testes at weaning was significantly decreased ($p<0.05$) in the treatment group male offspring compared to controls. Apoptosis in the testes was evaluated using terminal *d*UTP nick-end labeling (TUNEL) staining. In the treatment group male pups, percentages of TUNEL-positive tubules, TUNEL-positive cells per tubule, and apoptotic cell index were significantly increased ($p<0.05$) at PND 21 compared to controls. Both serum and testicular testosterone were significantly decreased ($p<0.05$) in the treatment group males at weaning (0.06 ± 0.02 ng/mL treated vs. 0.49 ± 0.22 ng/mL controls; and 1.48 ± 0.64 ng/g testis treated vs. 3.94 ± 1.33 ng/g testis controls, respectively). Both the mRNA level of testicular P450scc and the protein expression of testicular

P450scc were significantly decreased ($p<0.01$) at weaning. Maternal fenvalerate exposure during lactation had little effect on mRNA levels of testicular StAR, 17β-HSD, and $P450_{17\alpha}$, whereas protein expression of $P450_{17\alpha}$ in the testes was slightly decreased (not significant) at weaning.

In the adult offspring (PND 80), absolute and relative testes and prostate (with seminal vesicles) weights in males from mothers treated with fenvalerate during lactation were significantly decreased ($p<0.05$) by 11-25% compared to controls. There was no effect of fenvalerate on the weight of the epididymides of offspring adult males. Morphological analysis of the testes from the treatment group adult males at PND 80 showed 3/5 animals had testes with more than one large vacuole within the seminiferous tubules per testis cross-section. Moreover, 2/5 animals had testes that contained more than one seminiferous tubule with complete spermatogenic failure per testis cross-section. Exposure during lactation had no effect on the number of Leydig cells in the testes at adulthood. In addition, there was no effect on the percentages of TUNEL-positive tubules, TUNEL-positive cells per tubule, or apoptotic cell index in the testes of the offspring at adulthood. Both serum and testicular testosterone levels were unaffected in the treatment group adult males. Maternal fenvalerate exposure during lactation had little effect on the mRNA levels and protein expression of testicular StAR, 17β-HSD, $P450_{17\alpha}$, and P450scc in adult males.

The number of spermatozoa in the epididymides of the treated group adult males were significantly decreased ($p<0.05$; ↓18%). Fertility analysis showed 14/15 (93%) treated group males mated and 13/14 (93%) were fertile compared to 14/14 (100%) for the control in both parameters. There was no significant difference in the numbers of implantation sites, resorptions, live fetuses or dead fetuses per litter between the treated and control groups. In addition, no significant differences in the average litter size, male/female ratios, body weight, and crown length in the F2 generation were observed between the treated and control groups.

The study was conducted by the Department of Toxicology, Anhui Medical University (Hefei, China) and was reported according to the standards of the journal *Toxicology Letters*.

**CLASSIFICATION:** Not Applicable. This study does not conform to a recognized guideline requirement. The qualitative information provided in this study is considered to be acceptable for use during a weight-of-evidence evaluation of the OSRI.