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Reproductive and Developmental Toxicity of Amitraz in Sprague-Dawley Rats

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The present study was conducted to obtain information on the effects of amitraz on reproductive and developmental parameters in rats. The test chemical was administered via the drinking water containing 0, 40, 120, and 360 ppm to male rats from 2 weeks before mating to the end of 14-day mating period and to females from 2 weeks before mating, throughout mating, gestation and up to lactational day 4. During the study period, clinical signs, body weights, food intake, organ weights, reproductive and littering findings, necropsy findings, sperm parameters, and histopathology were examined. At 360 ppm, decreases in the body weight gain, food consumption, and the number of live pups and an increase in the post-implantation loss were observed. In addition, decreases in the seminal vesicle weight and sperm motility were found in males. At 120 ppm, a decrease in the food consumption was found transiently in both males and females, but no reproductive and developmental toxicity was observed in both sexes. There were no signs of either general or reproductive and developmental toxicity in the 40 ppm group. Based on these results, it was concluded that the repeated oral administration of amitraz to rats resulted in a decrease in the food consumption at 120 ppm and decreases in the seminal vesicle weight, sperm motility, and the number of live pups and an increase in the post-implantation loss at 360 ppm in rats. Under these experimental conditions, the no-observed-adverse-effect level (NOAEL) of amitraz for general and reproduction/developmental toxicity was believed to be 120 ppm, and the no-observed-effect level (NOEL) of amitraz was believed to be 40 ppm in rats.

Key words: Amitraz, Reproductive and developmental toxicity, Short-term screening test, Rats

INTRODUCTION

Amitraz, a formamidine insecticide and an acaricide, is widely used in fruit, cotton, and hops. It has also been available as a veterinary medicine in many countries since 1974 and mainly used for control of generalized demodicosis in dogs and ticks and mites on cattle and sheep. Incidences of amitraz poisoning in animals and humans have increased in recent years due to its increased production and use (Jorens *et al*., 1997; Proudfoot, 2003; Ulukaya *et al*., 2001). Amitraz poisoning can occur by inhalation, ingestion, and skin contact. Because amitraz is a pharmacologically active compound which has a_2-agonist actions, the most common adverse effects associated with amitraz are central nervous system alterations, such as depression, hypotension, polyuria, hyperglycemia, vomiting, and respiratory failure (Cullen and Reynoldson, 1987; Leung *et al*., 1999; Shin and Hsu, 1994).

The toxicity of amitraz has been studied extensively over the past several decades using both short- and long-term animal tests. The EPA classifies amitraz as class III-slightly toxic (EPA, 1987). The oral LD_{50} values for rats and mice are 400~938 mg/kg and above 1600 mg/kg body weight, respectively. The possible adverse effects of amitraz on the reproductive and developmental outcomes have also been examined using experimental animals. According to Palermo-Neto *et al*. (1994), the treatment of pregnant female rats with amitraz resulted in the opening of the vagina at an earlier age, as well as earlier fur development and delays in incisor eruption in the offspring. A subsequent study also showed that the postnatal exposure of lactating dams to amitraz caused transient developmental and behavioral changes in the exposed offspring (Palermo-Neto *et al*., 1997). Al-Thani *et al*. (2003) reported a decrease in the fertility index, sperm production, and the number of viable fetuses and an

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increase in the postimplantation loss at a dose level of 40 ppm in male Swiss mice exposed to subchronic levels of amitraz in their drinking water. Recently, Kim *et al*. (2006) reported that amitraz was embryotoxic and teratogenic in rats at a maternally toxic dose (10 mg/kg/day). However, these toxicology reports on amitraz were determined to be inadequate to assess the chemical, because they did not follow Good Laboratory Practice (GLP) or did not totally comply with specific testing guidelines.

The present study was conducted to obtain reliable information on the possible toxic effects in compliance with the OECD Test Guideline (1995). A reproduction and developmental toxicity screening test of amitraz was performed in rats, and the results of this study are reported in this article.

MATERIALS AND METHODS

Animal husbandry and maintenance. Male and nulliparous female Sprague-Dawley rats were obtained from a specific pathogen free colony at Bio Genomics Inc. (Seoul, South Korea) and used after quarantine and acclimatization. Animals were housed at a temperature of 23 ± 3°C and a relative humidity of 50 ± 10%. Artificial lighting was present from 8:00 a.m. to 8:00 p.m. and the room had 13~18 air changes per hour. All rats included in the study were healthy. Mating was achieved by placing 1 females and 1 male in a cage overnight, and successful mating was confirmed by the presence of sperm in vaginal cytology on the following morning. The first 24 h after mating was designated as day 0 of gestation (GD 0). Inseminated females were singly housed in clear polycarbonate cages with stainless steel wire lids and were given sterilized tap water and commercial rodent chow (Samyang Feed Co, Wonju, Korea) ad libitum. The Institutional Animal Care and Use Committee of Chonnam National University approved protocols for the animal study, and the animals were cared for in accordance with the Guidelines for Animal Experiments of Chonnam National University.

Test chemical and treatment. Amitraz was purchased from the Green Cross Veterinary Pharmaceutical Co. (Yongin, Korea). The test chemical was diluted to the appropriate concentration with tap water and prepared fresh daily before treatment. The control rats received tap water alone. The test chemical was administered via the drinking water containing 0, 40, 120, and 360 ppm to male rats from 2 weeks before mating to the end of 14-day mating period and to females from 2 weeks before mating, throughout mating, gestation and up to lactational day 4.

Experimental groups. Healthy males and females were assigned randomly into four experimental group; three treatment groups receiving 40, 120, and 360 ppm amitraz and a vehicle control group. Each group contained of 12 rats of each gender.

Selection of doses. Our previous subacute toxicity study showed that 4-week repeated administration of amitraz caused suppression of body weight gain at 300 ppm and no sign of general toxicity at 100 ppm (unpublished). Based on the results of previous study, 360 ppm was selected as the high dose and doses of 120 and 40 ppm were selected as middle and low doses, using a scaling factor of × 3.

Observation of parent animals. All animals were observed daily for clinical signs when treated with the test chemical, and then abnormal signs were recorded individually for type, observation day/time, and duration. Body weight was measured twice a week in each male rat until sacrifice. Female rats were weighed twice a week during the premating period and on gestational days (GD) 0, 3, 7, 14, and 21, and lactational days (LD) 4. The individual amount of food consumed was recorded twice a week during the study.

After the 2-weeks pre-mating exposure period was completed, the animals were mated on the basis of one male to one female, selected randomly within each dose group for a period of 14 days. The observation of vaginal plug and/or sperm in vaginal smear was considered evidence of successful mating. Females were examined daily during the mating period. The day a vaginal plug and/or sperm in vaginal smear were observed was designated as day 0 of pregnancy. Once the vaginal plug or sperm were observed, the female and male were separated and housed individually in a polycarbonate cage. Any female that did not show evidence of successful mating after cohabitation period was individually housed and was sacrificed on day 21 after separation. From GD 20, females were checked two times daily for evidence of onset, progress, and completion of parturition. All animals were allowed to litter naturally (F_1 generation), and rear their own offspring until LD 4. Dams were examined daily for evidence of normal maternal behavior. Based on these results, copulation, fertility, and pregnancy indices; pre-coital interval; and duration of pregnancy were calculated.

All animals were euthanized by ether inhalation and exsanguination from the aorta. A complete gross postmortem examination was then performed. The males were sacrificed at the end of the 14 day mating period while the delivered females were sacrificed on day 4 after parturition. The ovaries and uterus form each female were removed and examined corpora lutea number and implantation site status. The non-pregnant females were sacrificed on day 21 after the end of mating period and the uteri were removed. The uteri with no evidence of implantation were stained with 2% sodium hydroxide solution to identify the presence of early resorption sites (Yamada *et al*., 1985). If no stained implantation sites were present, the rat was considered not pregnant.

At the necropsy, male and female reproductive organs

(testes, epididymides, ventral prostates, seminal vesicles, and ovaries) were weighted. The right testis and epididymis in males and ovary in females were taken and fixed with 10% neutral buffered formalin solution. The tissues were routinely processed, embedded in paraffin and sectioned at 3~5 μm thickness, deparaffinized, and rehydrated using standard techniques. These sections were stained with Hematoxylin-Eosin for microscopic examination.

Sperm examination. Sperm analysis was conducted as previously described (Chung *et al*., 2005; Kim *et al*., 1999). At the scheduled necropsy, the testes and epididymides were removed and weighed. The left testis was homogenized with 12 *ml* of distilled water for the sperm head counts. The sperm suspension was placed into a hemacytometer (Neubauer, Germany) and the number of spermatids was counted using a light microscope (Leica, Germany). The left cauda epididymis was homogenized with 10 *ml* physiological saline to determine the sperm counts. The number of sperm was counted as in the testis. For the motility measurements, the sperm was obtained from the ductus deferens, placed in Hanks' balanced salt solution (pH 7.2, Sigma Chemical Co., St. Louis, MO, USA) containing 5 mg/*ml* bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) and maintained at 37°C. Motility was observed using a microscope with a stage warmer (Microwarm plat; MDF-10, Japan). The sperm morphology was also examined using optical microscopy of the sperm smears (sperm suspension containing 1% Eosin Y) collected from the left ductus deferens. Two hundred sperm per animal were evaluated for head and tail defects by light microscope. The sperm morphology was classified as normal, small head, amorphous head, two heads/tails, straight hook, excessive hook, folded tail, short tail, and no tail.

Observation of F1 animals. The morning on which parturition was complete was designated as postnatal day (PND) 0. All pups were examined on the day of birth to determine the number of live and stillborn pups in each litter. All live pups were individually counted, sexed, weighed, and examined external abnormalities and anogenital distance. Also, the number of live and dead pups was noted and all live pups were weighted on PND 4. The pups were euthanized by carbon dioxide inhalation and gross external, including palate, and internal examinations were performed on PND 4.

Statistical analysis. The data are presented as the mean ± SD. The unit for statistical measurement was a pregnant dam or a litter (Weil, 1970). If the data was parametric, it was subjected to one-way analysis of variance (ANOVA). Otherwise, the data were analyzed using Kruskal-Wallis non-parametric ANOVA (1952). If either of these tests showed statistical significance, the data was analyzed using the multiple comparison procedure reported by Dunnett (1964) or Scheffe (1953). The clinical signs and gross findings are presented as the frequencies, and were analyzed using Fisher's exact probability test (Fisher, 1970). The statistical analyses were performed using GraphPad InStat (Graph-Pad Software Inc. San Diego, CA) software. The significance of the differences between the groups was estimated at the probability levels of 1 and 5%.

RESULTS

Clinical sign. During the study period, no treatment-related mortality was noted among animals exposed to amitraz. However, the 360 ppm group showed treatment-related clinical signs after days 7 on test, such as a reddish tear (n = 5), nasal discharge (n = 4), fur staining (n = 4), dull fur (n = 5), and nervousness (n = 2) in males and a reddish tear (n = 8), nasal discharge (n = 5), fur staining (n = 8), dull fur (n = 5), and nervousness (n = 5) in females. Although these findings were also found in the middle dose group, the incidence and severity were low. No treatment-related clinical

Table 1. Body weight changes in male rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
No. of males	12	12	12	12
Day 0	228.2 ± 11.56[a)]	220.2 ± 8.66	221.7 ± 9.80	231.5 ± 8.37
Day 3	245.0 ± 14.21	237.7 ± 8.15	236.5 ± 9.16	232.3 ± 9.76*
Day 7	261.2 ± 15.89	252.8 ± 7.87	250.5 ± 11.48	231.8 ± 9.75**
Day 10	271.4 ± 17.65	263.8 ± 9.20	260.6 ± 12.15	233.2 ± 13.47**
Day 14	288.4 ± 22.41	279.8 ± 9.03	276.3 ± 12.31	252.2 ± 11.47**
Day 17	294.0 ± 22.87	287.1 ± 9.91	284.7 ± 13.70	258.5 ± 12.15**
Day 21	306.3 ± 26.60	297.9 ± 12.69	291.5 ± 16.80	266.6 ± 13.38**
Day 24	316.7 ± 27.51	307.2 ± 10.31	305.0 ± 18.78	274.4 ± 12.72**
Day 28	329.8 ± 29.15	318.9 ± 10.15	316.6 ± 19.98	279.8 ± 16.79**

[a)]Values are presented as mean ± SD.
*,**Significant difference at $p < 0.05$ and $p < 0.01$ levels compared with the control group, respectively.

findings were observed in either sex of the low dose group.

Body weight and food consumption. Table 1 and 2 show the changes in body weight in each group during the study period. The body weights on days 3-28 of treatment in the male 360 ppm group and GD 3 to LD 4 of treatment in the female 360 ppm group were significantly lower when compared with the control group. In contrast, there were no statistically significant changes in both genders of the 40 and 120 ppm groups. The results of food consumption are presented in Table 3 and 4. In males, the amount of food consumed was significantly lower on days 3-10 and 17-28 of treatment in the 360 ppm group and day 28 of treatment in the 120 ppm group than that of the control group. In females, food consumptions on days 0 and 3 of treatment, GD 3, and LD 3 in the 360 ppm group and days 3 and 10 of treatment in the 120 ppm group were significantly decreased when compared with the control group. In contrast, there were no statistically significant differences in both genders of the 40 ppm group.

Reproductive performance, pregnancy, and parturition findings. There were no adverse effects of treatment on the mating index, pre-coital time, fertility index, and

Table 2. Body weight change in female rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
Pre-mating period				
No. of females	12	12	12	12
Day 0	181.5 ± 10.17[a)]	181.7 ± 5.63	176.7 ± 14.03	181.9 ± 17.57
Day 3	188.7 ± 10.15	189.3 ± 7.46	185.5 ± 15.89	181.8 ± 19.14
Day 7	199.7 ± 12.40	198.9 ± 7.20	193.1 ± 18.28	190.0 ± 18.24
Day 10	206.8 ± 12.18	202.2 ± 7.81	196.9 ± 17.60	191.2 ± 18.39
Day 14	216.8 ± 14.16	211.1 ± 8.53	208.2 ± 18.91	198.2 ± 19.56
Gestation period				
No. of females	12	11	11	11
Day 0	219.2 ± 17.03	214.7 ± 10.66	205.5 ± 16.74	202.2 ± 21.42
Day 3	229.5 ± 16.29	222.1 ± 18.71	215.1 ± 20.79	202.9 ± 23.80**
Day 7	245.0 ± 15.34	237.7 ± 15.69	229.5 ± 16.31	214.1 ± 21.79**
Day 10	257.2 ± 16.10	250.2 ± 17.15	242.5 ± 20.88	224.7 ± 23.09**
Day 14	275.9 ± 16.75	268.2 ± 19.65	259.6 ± 21.56	239.8 ± 28.52**
Day 21	337.5 ± 21.41	332.4 ± 35.83	329.5 ± 29.46	282.1 ± 42.12**
Lactation period				
No. of females	12	11	11	11
Day 0	267.1 ± 14.13	255.0 ± 9.01	254.1 ± 21.26	237.6 ± 29.58**
Day 4	272.5 ± 21.54	262.6 ± 8.70	263.1 ± 22.66	239.3 ± 31.75**

[a)]Values are presented as mean ± SD.
**Significant difference at $p < 0.01$ level compared with the control group.

Table 3. Food consumption in male rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
No. of males	12	12	12	12
Day 0	16.25 ± 1.41[a)]	16.50 ± 1.09	15.67 ± 1.80	14.83 ± 1.78
Day 3	16.50 ± 0.43	15.92 ± 0.87	15.25 ± 1.53	9.92 ± 2.10**
Day 7	16.92 ± 1.02	16.08 ± 0.36	16.08 ± 1.58	11.25 ± 3.09**
Day 10	18.33 ± 1.30	18.17 ± 0.72	16.92 ± 1.10	12.75 ± 4.42**
Day 14	17.79 ± 3.76	16.08 ± 1.44	17.33 ± 2.18	15.25 ± 4.41
Day 17	19.46 ± 1.60	17.92 ± 1.00	17.33 ± 1.96	13.88 ± 4.94**
Day 21	22.42 ± 0.70	21.25 ± 0.72	21.25 ± 1.44	16.83 ± 1.30**
Day 24	20.75 ± 2.35	20.00 ± 1.13	20.25 ± 1.62	17.08 ± 0.93**
Day 28	21.33 ± 1.64	20.58 ± 1.74	18.75 ± 1.47**	18.75 ± 1.47**

[a)]Values are presented as mean ± SD.
**Significant difference at $p < 0.01$ level compared with the control group.

Table 4. Food consumption in female rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
Pre-mating period				
No. of females	12	12	12	12
Day 0	12.83 ± 1.44[a)]	12.67 ± 1.97	11.75 ± 1.08	10.50 ± 1.45**
Day 3	13.00 ± 1.31	13.41 ± 0.70	11.75 ± 0.99*	8.42 ± 1.14**
Day 7	13.92 ± 1.10	12.58 ± 1.36	14.00 ± 1.65	12.58 ± 1.64
Day 10	13.83 ± 1.87	12.17 ± 1.95	11.00 ± 2.47**	13.83 ± 1.27
Day 14	12.88 ± 1.23	12.33 ± 1.45	11.85 ± 1.47	12.83 ± 1.12
Gestation period				
No. of females	12	11	11	11
Day 0	12.50 ± 1.38	13.33 ± 2.99	12.18 ± 2.36	11.92 ± 3.58
Day 3	17.58 ± 1.68	16.00 ± 2.04	17.20 ± 2.74	13.08 ± 2.64**
Day 7	17.92 ± 1.31	18.08 ± 2.91	16.09 ± 3.27	15.67 ± 1.83
Day 10	17.67 ± 1.44	16.50 ± 2.68	17.91 ± 1.92	16.08 ± 3.48
Day 14	19.58 ± 1.44	17.00 ± 2.76	18.73 ± 4.17	17.42 ± 4.21
Day 21	20.58 ± 2.81	19.75 ± 3.62	21.18 ± 3.16	17.42 ± 4.34
Lactation period				
No. of females	12	11	11	11
Day 0	24.38 ± 2.72	24.13 ± 4.85	23.86 ± 6.98	20.25 ± 6.71
Day 4	40.72 ± 11.65	41.52 ± 6.01	39.73 ± 8.11	29.72 ± 9.14*

[a)]Values are presented as mean ± SD.
*,** Significant difference at $p < 0.05$ and $p < 0.01$ levels compared with the control group, respectively.

Table 5. Pregnancy and parturition findings of dams treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
No. of normal parturition	12	11	11	11
Gestation length (days)	21.71 ± 0.40[a)]	21.91 ± 0.44	21.86 ± 0.50	21.95 ± 0.52
No. of corpora lutea	13.83 ± 2.62	14.36 ± 2.25	15.45 ± 1.92	13.45 ± 1.63
No. of implantations	12.17 ± 1.90	13.45 ± 1.86	13.00 ± 1.34	11.18 ± 3.22
Pre-implantation loss (%)[b)]	11.16 ± 9.00	5.82 ± 7.92	14.80 ± 12.57	17.09 ± 22.46
No. of live pups at birth	10.92 ± 2.97	11.64 ± 3.35	12.00 ± 1.84	6.36 ± 3.59**
No. of stillborns	0	0.55 ± 1.81	0.18 ± 0.60	0.55 ± 1.29
Post-implantation loss (%)[c)]	11.23 ± 15.66	13.83 ± 1912	7.92 ± 7.86	39.67 ± 29.79**
Body weight of pups: male	6.83 ± 0.63	6.62 ± 0.60	6.60 ± 0.52	6.20 ± 0.44
females	6.38 ± 0.58	6.22 ± 0.50	6.11 ± 0.53	5.87 ± 0.46
Anogenital distance (mm): males	5.03 ± 0.30	5.09 ± 0.31	4.96 ± 0.30	4.77 ± 0.42
females	2.85 ± 0.15	2.80 ± 0.20	2.84 ± 0.17	2.77 ± 0.18
Sex ratio (male/female)	1.09 ± 0.74	0.76 ± 0.45	0.87 ± 0.46	0.61 ± 0.57
No. of pups with external anomalies (%)	0	0	0	1[d)]
Postnatal day 4				
No. of live pups	10.83 ± 2.92	11.64 ± 3.35	11.91 ± 1.92	6.27 ± 3.74**

[a)]Values are presented as mean ± SD.
[b)][(No. of corpora lutea – No. of implantation sites)/No. of corpora lutea] × 100.
[c)][(No. of implantation sites – No. of live embryos)/No. of implantation sites] × 100.
[d)]Torticollis.
**Significant difference at $p < 0.01$ level compared with the control group.

pregnancy index of either the males or females at any of the dose levels tested (data not shown). The results of pregnancy and parturition findings are presented in Table 5. The numbers of live pups at birth and PND 4 were significantly decreased when compared with the control group. In contrast, post-implantation loss was significantly increased in comparison with that of the control group. External anomalies in pups of rats given amitraz are also presented in Table

5. No fetuses with external malformations were observed in the control and groups given amitraz at 40 and 120 ppm. However, torticolis was observed in one pup in the 36o ppm group.

Necropsy and Organ weights. At the scheduled necropsy, No treatment-related gross lesions of the reproductive organs were noted in F0 males and females. As shown in Table 6, absolute weights of right testis, epididymides, ventral prostate, and seminal vesicles and relative weights of seminal vesicles in the male 360 ppm group were significantly decreased in comparison with those of the control group. In females, no significant difference was found in the absolute and relative weights of ovaries between the control and treatment groups.

Sperm examination. The results of sperm exanmination are presented in Table 7. Sperm motility in the 360 ppm group was significantly decreased when compared with the control group. Although sperm head count in testis and sperm count in caudal epididymis were slightly decreased when compared to control group, the differences were not

Table 6. Absolute and relative reproductive organ weights (g) of male and female rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
No. of males	12	12	12	12
Testis: right	1.5 ± 0.11	1.52 ± 0.10	1.49 ± 0.08	1.40 ± 0.12*
per body weight (%)	0.46 ± 0.04	0.48 ± 0.04	0.47 ± 0.03	0.51 ± 0.05
Testis: left	1.56 ± 0.13	1.56 ± 0.12	1.49 ± 0.08	1.46 ± 0.06
per body weight (%)	0.47 ± 0.04	0.49 ± 0.04	0.47 ± 0.03	0.53 ± 0.03
Epididymis: right	0.54 ± 0.03	0.53 ± 0.04	0.52 ± 0.04	0.48 ± 0.03**
per body weight (%)	0.17 ± 0.01	0.17 ± 0.02	0.16 ± 0.01	0.17 ± 0.01
Epididymis: left	0.56 ± 0.04	0.55 ± 0.02	0.53 ± 0.04	0.50 ± 0.05**
per body weight (%)	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.01	0.18 ± 0.02
Ventral prostate	0.70 ± 0.13	0.74 ± 0.10	0.68 ± 0.11	0.52 ± 0.08**
per body weight (%)	0.21 ± 0.03	0.23 ± 0.03	0.21 ± 0.04	0.19 ± 0.03
Seminal vesicles	1.38 ± 0.14	1.36 ± 0.11	1.24 ± 0.21	0.92 ± 0.20**
per body weight (%)	0.42 ± 0.04	0.43 ± 0.03	0.39 ± 0.07	0.33 ± 0.07**
No of females	12	11	11	11
Ovary: right	0.063 ± 0.008	0.062 ± 0.006	0.059 ± 0.004	0.057 ± 0.007
per body weight (%)	0.023 ± 0.006	0.024 ± 0.005	0.023 ± 0.003	0.026 ± 0.005
Ovary: left	0.060 ± 0.007	0.062 ± 0.004	0.058 ± 0.006	0.055 ± 0.008
per body weight (%)	0.022 ± 0.006	0.024 ± 0.005	0.023 ± 0.007	0.025 ± 0.005

[a)]Values are presented as mean ± SD.
[*,**]Significant difference at $p < 0.05$ and $p < 0.01$ levels compared with the control group, respectively.

Table 7. Results of sperm analysis in male rats treated with amitraz via drinking water

Items	Amitraz in water (ppm)			
	0	40	120	360
No. of males	12	12	12	12
Sperm head count (× 10^6/testis)	246.4 ± 24.86[a)]	234.6 ± 24.88	235.3 ± 22.31	224.2 ± 15.13
Sperm count (× 10^6/caudal epididymis)	209.1 ± 18.76	204.4 ± 12.37	200.6 ± 21.85	195.4 ± 25.44
Sperm motility (%)	75.7 ± 7.05	77.8 ± 11.81	69.5 ± 10.77	63.3 ± 10.07*
Sperm abnormalities (%)	6.8 ± 3.41	6.7 ± 2.87	5.8 ± 1.90	7.8 ± 3.98
Small head (%)	0.2 ± 0.58	0	0.2 ± 0.58	0.2 ± 0.58
Amorphous head (%)	0.5 ± 0.80	0.7 ± 1.15	0	0.7 ± 0.78
Two head/tail (%)	0.3 ± 0.49	0.3 ± 0.49	0.2 ± 0.39	0
Excessive hook (%)	0.2 ± 0.39	0.3 ± 0.78	0	0.1 ± 0.29
Blunt hook (%)	0.8 ± 0.39	1.8 ± 1.75	0.2 ± 0.39	0.2 ± 0.39
Folded tail (%)	4.3 ± 1.97	3.0 ± 1.60	3.8 ± 1.95	4.7 ± 2.67
Short tail (%)	0	0	0.2 ± 0.39	0.2 ± 0.39
No. tail (%)	0.5 ± 0.80	0.5 ± 0.80	1.3 ± 0.78	1.8 ± 1.64

[a)]Values are presented as mean ± SD.
[*]Significant difference at $p < 0.05$ level compared with the control group.

statistically significant between the groups.

Histopathological examination. Degeneration of spermatocytes in testis and exfoliation of degenerative germ cells in epididymal ducts were observed in 1 male each in the 360 ppm group. No histopathological changes were observed in ovaries at any dose tested.

DISCUSSION

The present study was carried out to investigate the potential adverse effects of amitraz on reproductive and developmental parameters in rats. The results of the current study showed that short-term repeated oral dose of amitraz to rats caused reproductive and developmental toxicity at 360 ppm.

Clinical signs observed in the 360 ppm group, such as a reddish tear, nasal discharge, fur staining, dull fur, and nervousness, were considered to be related to the amitraz treatment because these findings were observed throughout the treatment period and showed a dose-response relationship. Of the adverse clinical signs, the increased incidence of nasal discharge and nervousness might be the result of the á$_2$-adrenergic effect of amitraz. Some clinical signs, such as a reddish tear, fur staining, and dull fur, suggest stress as a result of exposure to the test chemical. According to the previous reports (Cullen and Reynoldson, 1987; Leung *et al*., 1999), the most common adverse effects in human associated with amitraz are central nervous system alterations, such as depression, hypotension, polyuria, hyperglycemia, vomiting, and respiratory failure.

The significant suppression of body weight observed in both genders of the 360 ppm group was considered to be related to the amitraz treatment since this finding showed a clear-cut dose-dependent relationship and was consistent with the increased clinical signs and decreased food consumption in this group. This is a clear indication of the general toxicity induced by amitraz, which suggests that oral exposure to this chemical cause mild anorexia, followed by the suppression of body weight gain in rats. On the contrary, the significant decrease of food consumption in both genders of the 120 ppm group was of no toxicological significant as the change was observed transiently and unaccompanied by correlated findings.

With regard to reproductive parameters, no adverse effects of amitraz on mating, fertility, and pregnancy indices and pre-coital time were observed. According to the previous studies, administration to male rats of chlordimeform, a formamidine insecticide, caused decreases in concentrations of testosterone and gonadotropin releasing hormone (Goldman *et al*., 1990; Cooper *et al*., 1999), and a single intraperitoneal injection of chlorodimeform at ≥ 25 mg/kg caused delayed ovulation due to the inhibition of luteinizing hormone secretion (Goldman *et al*., 1990, 1991; Cooper *et al*., 1999). Recently, Al-Thani *et al*. (2003) reported that 12-week repeated oral dose of amitraz at 40 ppm showed decreases in fertility, sperm production, and the number of live pups and an increase in post-implantation loss. These discrepancies among the studies may be explained by the differences in test article, animal species, dose level, and dosing form. Further studies will be needed to determine the precise cause-effect relationship.

The developmental toxicity included decreased number of live pups at birth and PND 4 and increased post-implantation loss. The decrease in the number of live pups observed in the high-dose group is closely related to the administration of amitraz because this finding is in good agreement with the increased post-implantation loss. The significant increase in the post-implantation loss suggests that an interruption of pregnancy occurred during the embryo-fetal developmental stage, but not in the germinal developmental stage. At birth, a single pup in the high dose groups exhibited torticolis. However, it is considered incidental finding because the incidence was within the limits of the historical control rats (Morita *et al*., 1987; MARTA, 1997; Kim *et al*., 2001).

In general toxicity studies, it is well known that the body weight and organ weight are sensitive indicators of potentially toxic chemicals (Anderson *et al*., 1999; Baily *et al*., 2004). As described above, the administration of amitraz to pregnant/lactating rats caused a significant suppression in body weight in the 360 ppm group, indicating that the dose of 360 ppm amitraz is maternally toxic to rats. The suppressed body weight in the groups affected the absolute weights of some organs such as the testis, epididymis, and ventral prostate. However, the weight changes in the above organs are of uncertain toxicological significance because they are considered to be the result of a body weight reduction. In contrast, the significant decrease in the absolute and relative weights of seminal vesicles observed in the high dose group is considered to be treatment-related effects, since the change was remarkable and showed a clear-cut dose-response relationship. The histopathological changes observed in the high dose group were not considered to be related to treatment, because such changes are well known to occur commonly in normal Sprague-Dawley rats (Boorman, 1990; Greaves, 1990; Haschek and Rousseaux, 1998).

The significant decrease in sperm motility observed in the 360 ppm group was also considered to be related to the amitraz administration because this finding showed a dose-response relationship. Al-Thani *et al*. (2003) also reported a decrease in the fertility index, sperm production, and the number of viable fetuses and an increase in the post-implantation loss at a dose level of 40 ppm in male mice exposed to subchronic levels of amitraz in their drinking water.

In conclusion, the repeated oral administration of amitraz to rats resulted in a decrease in the food consumption at 120 ppm and decreases in the seminal vesicle weight, sperm

motility, and the number of live pups and an increase in the post-implantation loss at 360 ppm in rats. Under these experimental conditions, the no-observed-adverse-effect level of amitraz for general and reproduction/developmental toxicity was believed to be 120 ppm, and the no-observed-effect level of amitraz was believed to be 40 ppm in rats. The present results are expected to provide some information on the general and reproductive/developmental toxicity of amitraz, which can aid in the process of risk assessment.

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