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Fipronil Developmental Neurotoxicity Study OPPTS 870.6300

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DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - rat; OPPTS 870.6300

DP BARCODE: D228360 SUBMISSION CODE: S506080
P.C. CODE: 129121 TOX CHEMICAL NO.:

TEST MATERIAL (PURITY): Fipronil (96.1% a.i.)

SYNONYMS: MB 46030

CITATION: Mandella, R. C. (1995) A Developmental Neurotoxicity Study of Fipronil in the Rat via Dietary Administration. Pharmaco LSR (Huntingdon Life Sciences as of 11/21/95), East Millstone, NJ. Laboratory Report No. 93-4508, December 28, 1995. MRID 44039002. Unpublished

SPONSOR: Rhone-Poulenc

EXECUTIVE SUMMARY:

In a developmental neurotoxicity study (MRID 44039002), fipronil (96.1% a.i.) was administered to 30 female Sprague-Dawley rats/group in the diet at dose levels of 0, 0.5, 10 or 200 ppm (0.05, 0.90 or 15 mg/kg/day, respectively) from Gestation Day 6 to Lactation Day 10.

There was no evidence of a treatment-related effect on maternal survival or clinical signs of toxicity. Two females in the 200 ppm group died during lactation, but there was no evidence that the deaths were treatment-related.

Mean maternal body weight values for the 200 ppm group were reduced 15.5%, 10.0% and 8.6% in comparison to the controls on Gestation Days 10, 15 and 20, respectively. Mean body weight gain was statistically decreased for Gestation Day interval 6-10, but increased for interval 10-15. Statistically significant reductions in mean body weight were seen in the 200 ppm group on Lactation Days 0 and 4. Mean body weight gain was statistically increased on Lactation Days 4-11. A statistically significant reduction in group mean food consumption was noted in the 200 ppm group for Gestation Days 6 to 10 but was comparable to the

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controls for other intervals.

Pregnancy rate and gestation length for treated animals were comparable to the control group. There was no evidence of a treatment-related effect on gross necropsy findings.

The maternal LOEL is 200 ppm (15 mg/kg/day), based on decreased body weight, body weight gain and food consumption. The maternal NOEL was 10 ppm (0.90 mg/kg/day).

At 200 ppm, litter size was not affected by treatment, but the live birth index was decreased (not statistically significant). The pup viability index (survival from Postnatal Days 0-4) for the 200 ppm group was significantly decreased (98.9% for control vs. 75.5% for 200 ppm group). The weaning index (survival from Postnatal Days 4-21) was decreased for this group, but the difference was not statistically significant. Pup sex distribution was not affected. There was a statistically significant decrease in group mean body weights of both males and female offspring at all recorded intervals during lactation (9.2-34.1% and 8.1-33.8% decrease in males and females, respectively) and for various periods post-weaning. Statistically significant increases in the mean day of achieving pinna detachment, upper and lower incisor eruption, vaginal patency and preputial separation were noted. Auditory startle testing on Postnatal Day 22 demonstrated a statistically significant decrease in the maximum response for males and females. There was no significant difference in the time to maximum response or average response. There were no changes in this parameter on Postnatal Day 60. Motor activity testing on Postnatal Day 17 showed statistically significant increases in motor activity counts for females. Swimming direction scores on Day 6 were reduced for the males and females, although only the males were statistically significant. On Day 14, the scores were comparable. Water "Y" maze time trials for learning and memory showed a statistically significant increase in time required to complete the maze for females in Trials 5 and 6 on Day 24. There were no statistically significant differences for either sex on Days 25, 30, 60, 61 or 65. Statistically significant decreases in absolute brain weights for both sexes, compared to control values, were found on Postnatal Days 11 (20% and 11% decrease in males and females, respectively) and 60 (~ 7% decrease in males and females). Terminal body weights were also decreased for this group on these days. On Day 11, the relative brain weights for both sexes were significantly increased in comparison to the controls. On Day 60, the values for the control and 200 ppm groups were comparable. There was no evidence of a treatment-related effect on the gross macroscopic or microscopic examinations (including the central and peripheral nervous systems) of the pups sacrificed on Postnatal Days 11 and 60.

At 10 ppm, group mean weights were significantly reduced for

females at all recorded intervals and for males on Days 4 and 17. Post-weaning weights were not affected. There was a statistically significant increase in the time of preputial separation in the 10 ppm group males. Females had a statistically significant increase in mean motor activity counts on Postnatal Day 17.

The developmental LOEL is 10 ppm (0.9 mg/kg/day), based on statistically significant decrease in group mean pup weights during lactation and significant increase in time of preputial separation in males. The developmental neurotoxicity LOEL is 10 ppm (0.9 mg/kg/day) based on a significant increase in mean motor activity counts in females on Postnatal Day 17. The NOEL for developmental and developmental neurotoxicity is 0.5 ppm (0.05 mg/kg/day). It is noted that developmental neurotoxicity occurred in the absence of maternal toxicity in this study.

The developmental neurotoxicity study in the rat is classified **acceptable/guideline** and **does satisfy** the guideline requirement for a developmental neurotoxicity study (OPPTS 870.6300) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Fipronil
   Description: fine white powder
   Lot/Batch #: 6ADM93
   Purity: 96.1% a.i.
   CAS #: 120068-37-3

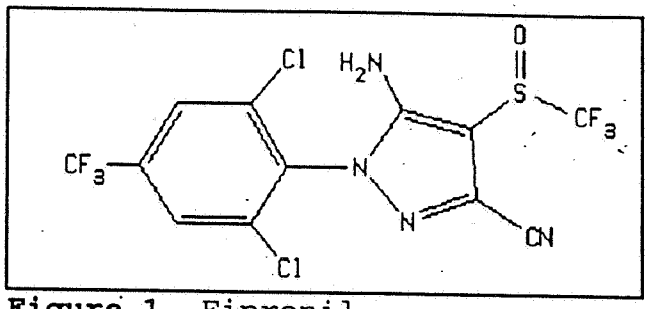

Figure 1 Fipronil

2. Vehicle: Acetone was used as a carrier to facilitate mixing of the test material; control animals received diet treated with acetone.

3. Test animals: Species: rat
   Strain: Sprague-Dawley derived (CD)
   Age at mating: 64 days
   Weight at mating: mean - 258.0 g; range 208.3 - 320.6 g
   Source: Charles River Breeding Laboratories, Portage, MI
   Housing: mated females were housed individually in suspended stainless steel cages, except during mating and during lactation; neonates were housed with dam during the lactation period; littermates of the same sex were housed together for one week following weaning and then individually
   Diet: Purina Certified Rodent Chow Brand Animal Diet #5002 ad libitum
   Water: municipal water by automatic watering system ad libitum
   Environmental conditions:
      Temperature: 67 - 75°F
      Humidity: 32 - 77%
      Air changes: Not provided
      Photoperiod: 12 hrs dark/12 hrs light
   Acclimation period: 14 days

B. PROCEDURES AND STUDY DESIGN

1. In life treatment dates - start: March 14, 1994; end: May 3, 1994

2. Mating: females selected for mating were placed with male rats nightly in a 1:1 ratio. Vaginal smears were taken the next day. If sperm were found on the smear or if a vaginal plug was observed, this was considered evidence of mating and defined as Day 0 of gestation.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment to groups was made daily to equalize, as best possible, the Day 0 gestation mean body weights.

TABLE 1 Animal Assignment

Test Group	Dose (ppm)	Treatment Schedule	Number of Females
Control	0	Gestation Day 6 to Lactation Day 10	30
Low (LDT)	0.5	Gestation Day 6 to Lactation Day 10	30
Mid (MDT)	10	Gestation Day 6 to Lactation Day 10	30
High (HDT)	200	Gestation Day 6 to Lactation Day 10	30

4. Dose selection rationale: The study report does not include the rationale for dose selection.

5. Diet preparation and analysis

The test formulations were prepared by mixing appropriate amounts of test substance with the basal diet; acetone was used as a carrier to facilitate mixing. The study report does not state how frequently the diets were prepared. Prior to the start of the study, mock-batches of the low- and high-concentration diets were prepared for homogeneity and stability analyses. The preparations were analyzed for stability after storage for 7, 14, 21 (low concentration) and 22 (high concentration) days. All three dietary levels for each mix prepared during the study were analyzed for concentration.

Results (Appendix R, page 466) -

Homogeneity Analysis: Homogeneity analyses on the mock-batches of the low and high concentration diets demonstrated that the mixing procedures were adequate. However, the low concentration diet was not within the ± 15% range of the nominal concentration. Subsequent diets fed to the animals were within the acceptable range of nominal.

Stability Analysis: mean % recovery for the 0.5 ppm diet was 120% on Day 1 and 108% on Day 21/22; mean % recovery for the 200 ppm diet was 97.6% on Day 1 and 93.3% on Day 21/22.

Concentration Analysis: 0.5 ppm - 100 ± 8.40% of nominal; 10 ppm - 98.7 ± 1.52% of nominal; 200 ppm - 97.7 ± 2.21% of nominal

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: All doses were administered in the diet, on gestation day 6 through lactation day 10.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality and clinical signs of toxicity twice daily. Mated females were given a physical examination on Days 0 and 6-21 of gestation. Females with litters were examined on Days 1-10, 14 and 21 of lactation. After weaning, weekly examinations were given. Body weight and food consumption data were recorded on gestation days 0, 6, 10, 15 and 20. On lactation days 0, 4, 11 and 21, females with litters were weighed. Dams were sacrificed following the weaning of the last litter. Gross examinations were conducted on all females found dead or sacrificed at the end of the study; abnormal tissues noted during the examination were fixed in 10% formalin.

2. Offspring Evaluations - The offspring were examined in the following manner:

A. In-life evaluations

Litters were observed as soon as possible after delivery (Day 0 of lactation) for number of live and dead pups and for any abnormalities. Thereafter, the litters were observed twice daily. Pups were counted and sexed on Days

0, 4 (pre- and post-cull), 11, 17, and 21 of lactation. Pup body weights were taken on Days 0, 4, 11, 17 and 21 of lactation and weekly post-weaning.

Developmental landmarks, including pinna detachment, eye opening and incisor eruption, were noted on the lactation day they were completed. The day on which vaginal patency (females) and preputial separation (males) occurred was recorded for animals retained post-weaning.

The motor activity of one male and one female in each litter was measured on Postnatal days 13, 17, 22 and 60 (±2). A Photobeam Activity System monitored activity for 60 minute sessions (12 5-minute intervals).

An auditory startle habituation test was performed on one male and one female pup/litter on Postnatal days 22 and 60 (±2). The response was measured using a Startle Response Screening System. The mean response in the presence and absence of a prepulse stimulus was determined and recorded using blocks of 10 trials (5 blocks of 10 trials/session).

Swimming development (direction, angle and paddling) were evaluated for one male and one female pup in each litter on postnatal days 6, 8, 10, 12 and 14. Each pup was given a 15 second trial.

Learning and memory were assessed for one male and one female pup per litter on Postnatal days 24 and 60. To measure learning, each rat was given six consecutive trials in a water Y-maze; the time required to find the correct arm of the Y-maze was recorded (acquisition phase of learning). To measure short- and long-term memory, each animal was evaluated for two more trials on Postnatal days 25, 30, 61 and 65.

B. Post-mortem Evaluations (inhaled carbon dioxide used for euthanasia unless otherwise noted)

F_1 generation dead pups: pups found dead at birth and during Day 0-4 of lactation were weighed and given a gross external and internal examination, including sex determination. They were eviscerated and preserved intact in 95% ethanol. Those found dead after Day 4 of lactation were examined similarly. Abnormal tissues were preserved in 10% neutral buffered formalin.

F_1 culled pups: on Day 4 of lactation, all litters with more than 8 pups were reduced to that number with equal numbers/sex when possible. These pups were given a

macroscopic external and internal examination, including sexing, and then discarded.

On Day 11 of lactation, one male or one female pup/litter was selected at random for sacrifice. Gross postmortem examinations were conducted and any abnormal tissues were saved. Brain weights were recorded and the organs were preserved in 10% formalin. Brain/terminal body weight ratios were calculated. Six pups/sex/dose group from the group sacrificed for brain weights were selected for neuropathological evaluation. Fixed brains from the control and high-dose group were embedded in paraffin, sectioned and stained with hematoxylin and eosin, Luxol Fast Blue and Sevier-Munger stain. Sections were taken through the cerebral cortex, hippocampus, basal ganglia, mid-brain, brainstem and cerebellum.

On Day 60 post-partum, one male or one female from each litter was randomly selected for sacrifice. Gross necropsy examinations were conducted and abnormal tissues were saved. Brain weights were recorded and the organs were preserved in 10% formalin. Brain/terminal body weights ratios were calculated.

On Day 60 post-partum, six animals/sex/dose group were selected randomly and sacrificed for neuropathological evaluation. Animals were anesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 4% paraformaldehyde in the same buffer. Gross necropsy examinations were performed. Following perfusion, the brain, spinal cord, and three peripheral nerves (sciatic, tibial and sural nerves) were removed and placed in 4% paraformaldehyde in phosphate-buffered saline for 24 hours and then prepared for microscopic examination. Sections of the following tissues (stained as indicated for Day 11) from the control and high-dose groups were examined: cerebral cortex, hippocampus, basal ganglia, mid-brain, brainstem, cerebellum and spinal cord (cervical, thoracic and lumbar areas). The peripheral nerves were embedded in plastic, sectioned at one micron and stained with toluidine blue. The tissues were examined for any evidence of spontaneous, technique-related or treatment-related changes. Simple visual assessment was made by examining the slides macroscopically to compare regions of the brain and spinal cord in the control and high dose groups. Microscopically, a visual comparison was made of the thickness of major areas within the neocortex, hippocampus and cerebellum. Measurements were not recorded unless there appeared to be differences between the treated and control animals.

Following completion of all evaluations, remaining animals were sacrificed. Gross examinations were conducted and abnormal tissues were preserved in 10% formalin.

D. DATA ANALYSIS

1. Statistical analyses: Interval data (mean body weight and body weight change, mean food consumption, mean gestation length, mean number of pups, mean pup weight, mean pup live birth, viability and weaning indices, mean learning and memory data) were first analyzed for equal variance using the Bartlett's test. If the variances were equal, the standard one-way ANOVA using the F distribution was used to assess significance. If significant differences were indicated, Dunnett's test was used to determine which means were significantly different from the control. If the variances were not equal, the Kruskal-Wallis test was used and if differences were indicated, a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed.

Incidence data (mortality rate, pregnancy rate and litter survival) were analyzed using contingency tables. First, a chi-square test was performed to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2x2 Fisher Exact Test. Third, the Armitage's test for linear trend in dosage groups was performed.

The four non-sexual developmental landmarks were analyzed by two methods. First, a linear regression of the logit transformed proportion of animals achieving the criterion on each day was performed. The independent variables were dose group, day of achievement and the interaction of these variables. The second analysis was based on the multivariate profile analysis using dose group as the independent variable and accounting for the serial correlation in the successive proportions of animals achieving the criterion.

The day of observing the sexual development landmark was analyzed by the Cox proportional hazards regression model. A second analysis was performed on these data to account for intralitter correlation.

The three startle responses were analyzed by a profile repeated measures design. Pairwise contrasts comparing

each treated group to control were considered when the overall main effect was statistically significant.

Motor activity data were transformed by Blom's inverse normal transformation to help achieve normality of the model residuals. The analysis was a profile analysis across testing days and intervals within the testing days. Pairwise contrasts comparing each treated group to control were considered when the overall main effect was statistically significant.

The swimming development analysis was a cumulative logit model, with treatment time as a repeated factor.

2. Historical control data: Historical control data (Appendix U, page 501) were provided to allow comparison with concurrent controls.

3. Positive control data: Data were submitted for the following chemicals (doses): hydroxyurea (550 mg/kg/day), methylmercury (II) chloride (6 mg/kg/day) and 5,5-diphenylhydantoin sodium (150 mg/kg/day). It appears the data were from one study (labeled Study A). The endpoints evaluated were the same as those in the present study, except the sexual development endpoints (vaginal patency and preputial opening) were not included. The auditory startle response data measured mean response with stimulus, mean response with no stimulus and the difference. These measurements differ from the endpoints (maximum response, time to maximum response and average response) determined in the present study. In addition, motor activity evaluations were done on Days 13, 17, 22 and 42, as compared to Days 13, 17, 22 and 60 in the present study.

Only hydroxyurea showed an effect for the non-sexual development endpoints when compared to the mean of values for the vehicle control chemicals (identified only by numbers and referred to under Historical Control). For the water "Y" maze time trial results, the scores of the three vehicles were averaged by the EPA reviewer and compared to the results of the three positive controls. Males and females receiving hydroxyurea had consistently increased scores as compared to the controls, along with males receiving 5,5 diphenylhydantoin sodium. For the swimming development evaluations, similar calculations were done. Males and females receiving hydroxyurea and 5,5 diphenylhydantoin sodium had consistently lower scores than the controls for direction, paddling and angle. For motor activity on Days 13, 17 and 22, the direction of the scores (increased or decreased in comparison to the vehicle controls) for the positive

control males were not consistent. The females treated with hydroxyurea had consistently lower motor activity scores than the vehicle controls, whereas females treated with the other two positive controls had consistently higher scores.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: Two females in the 200 ppm group died during lactation (on days 6 and 9). The animals were not observed to be ill prior to death. The only observation on necropsy was discoloration of the lungs. The study report states the deaths were not attributed to fipronil administration. There were no treatment-related clinical signs of toxicity in the remainder of the animals, except for alopecia (general and extremities/snout) which was increased in the 200 ppm group.

2. Body Weight - Body weight data are summarized in Table 2 and as follows: Mean body weight was reduced in the 200 ppm group during the treatment period (decreased 15.5%, 10.0% and 8.6% on Gestation Days 10, 15 and 20, respectively). The study report states that mean body weight gain was reduced for the Gestation Day 6-20 interval for this group, however these data were not presented in the tables for this parameter. The difference was due to significant body weight loss on Gestation Days 6-10. During the Gestation Day 10-15 interval, the 200 ppm group females had a statistically significant increase in body weight gain.

   During lactation, mean body weight of the 200 ppm group was significantly reduced 12 and 11% on Lactation Days 0 and 4, respectively. However, during Lactation Days 4-11 (the period immediately following standardization of litter size), there was a statistically significant increase in body weight gain. When fipronil treatment was terminated on Lactation Day 10, body weight gain for this group was comparable to control weight gain. On Lactation Day 21, mean body weight for the 200 ppm group females was comparable to the control group.

   Mean body weight and body weight gain for the 0.5 and 10 ppm groups were comparable to the control group during gestation and lactation.

TABLE 2 Maternal Body Weight Gain (g)[a]

Interval	Dose in ppm (# of Dams)			
	Control	0.5	10.0	200
Gestation - Body Weight				
Day 0	257 (27)	260 (29)	254 (29)	257 (29)
Day 6	297 (27)	303 (29)	293 (29)	297 (29)
Day 10	316 (27)	322 (29)	311 (29)	267** (29)
Day 15	351 (27)	357 (29)	346 (29)	316** (29)
Day 20	429 (27)	436 (29)	422 (29)	392** (29)
Gestation - Body Weight Gain				
Days 0-6	40 (27)	43 (29)	39 (29)	40 (29)
Days 6-10	19 (27)	19 (29)	18 (29)	-29** (29)
Days 10-15	35 (27)	35 (29)	35 (29)	49** (29)
Days 15-20	77 (27)	79 (29)	76 (29)	75 (29)
Lactation - Body Weight				
Day 0	329 (27)	338 (29)	322 (29)	291** (29)
Day 4	348 (27)	352 (29)	339 (29)	308** (25)
Day 11	353 (27)	359 (29)	351 (29)	343 (23)
Day 21	355 (27)	363 (29)	358 (29)	355 (23)
Lactation - Body Weight Gain				
Days 0-4	18 (27)	14 (29)	18 (29)	13 (25)
Days 4-11	6 (27)	7 (29)	12 (29)	35** (23)
Days 11-21	2 (27)	3 (29)	7 (29)	12 (23)

a Data extracted from Tables 3 (pages 61-62) and 6 (pages 65-66).
** Statistically different from controls, $p<0.01$

3. Food Consumption - Food consumption data are summarized as follows: Mean food consumption was decreased by approximately 50%, relative to control values, for the 200 ppm group for the Gestation Day 6-10 interval. Values were comparable to the control for the remainder of the gestation period. The 0.5 and 10 ppm groups were comparable to the controls. Food consumption was not measured during lactation.

4. Test Substance Intake

The range of test substance intake in mg/kg/day for maternal females, calculated from food consumption data and based on nominal concentrations, were as follows:

Group	Dose (ppm)	Range (mg/kg/day)
II	0.5	0.05
III	10	0.90 - 0.92
IV	200	8.73 - 18.49

For the high dose animals, the minimal intake of 8.73 mg/kg/day was determined for the period of gestation days 6-10, when food consumption was reduced 50% and the dams lost weight. The study report describes these intake levels as: 0.05, 0.9 or 15 mg/kg/day for the low-, mid- and high-dose groups, respectively.

5. Pregnancy Status and Litter Data (see Table 3) - Pregnancy rate for the control group was 90.0%; the rate for all treated groups was 96.7%.

Mean gestation length was not affected by fipronil treatment (22.1 days for the control vs. 22.0 days for the 200 ppm group).

The mean number of total pups born and the mean number of live pups born were not affected by treatment. There was a statistically significant increase in the number of dead pups born for the 200 ppm group when compared to the control group (0.1 for the control group vs. 1.1 for the 200 ppm group). However, since there was a greater total number of pups born in 200 ppm group, the increase in dead pups did not result in a decrease in the mean number of pups born alive. The mean pup live birth index[1] for the 200 ppm group was lower than the control, but the difference was not statistically significant.

The ratios of male pups to female pups was comparable to the control ratios for all the treatment groups.

[1] Live birth index = $\frac{\text{total pups born alive}}{\text{total pups born}} \times 100$

Table 3: Litter Data[a]

Observation	Treatment Level (ppm)			
	0	0.5	10	200
Number Mated	30	30	30	30
Number Pregnant (%)	27 (90.0%)	29 (96.7%)	29 (96.7%)	29 (96.7%)
Mean Gestation Length (days)	22.1	22.0	22.0	22.0
Mean Total Pups Born	14.9	15.5	15.6	15.9
Mean Pups Dead at Birth	0.1	0.2	0.2	1.1**
Number of Stillbirths	4	5	7	32
Mean Live Birth Index	99.1	98.9	98.4	93.0
Sex Ratio (M:F, Lactation Day 0)	1.1	1.0	1.0	1.1
Mean Number of Pups Alive				
Day 0	14.7	15.3	15.3	14.8
Day 4 (pre-cull)	14.6	14.7	15.0	12.9*
Day 4 (post-cull)	7.9	7.9	8.0	7.9
Day 11	7.9	7.9	8.0	7.5
Day 17	6.9	6.9	7.0	6.5
Day 21	6.9	6.9	7.0	6.5

a Data extracted from Tables 7 and 8 of the study report
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

B. PUP TOXICITY

1. Pup Weight and Weight Gain - Pup weight during lactation and postweaning is summarized in Tables 4a and 4b, respectively. Preweaning pup weights for the 10 and 200 ppm groups were reduced in comparison to the control group. Group mean weights were reduced for the 200 ppm group males and females throughout the lactation period and for some of the post-weaning intervals (two for males and three for females). Pups in this group also did not gain as much weight during the lactation period as the controls. (Actual body weight gain data for pups were not calculated.)

Group mean weights for the 10 ppm group females were statistically significantly reduced for females at all recording intervals during lactation and for males on Days 4 and 17. Group mean pup weight gains for this group during lactation were comparable to the controls.

There was a statistically significant decrease in group mean pup weight of females in the 0.5 ppm group on Day 4 post-cull. The study report states that this decrease was not considered toxicologically significant since the decrease was slight and values were the same as the body weight values on Day 4 pre-cull.

During the postweaning period, which extended to termination of the offspring, mean body weight values were decreased for both males and females in the 200 ppm group for the entire duration of approximately seven weeks, with significant reductions in mean body weight noted at approximately Weeks 4 and 5 postweaning (8-12 weeks of age) for males and Weeks 3 through 5 (7-12 weeks of age) for females.

Table 4a: Mean Pup Weights During Lactation (g)[a]

	Fipronil Treatment Level (ppm)			
	0	0.5	10	200
Males				
Day 0	6.5	6.5 (100.0)[b]	6.3 (97.0)	5.9** (90.8)
Day 4 (Pre-cull)	10.7	10.4 (97.2)	10.0* (93.5)	7.7** (72.0)
Day 4 (Post-cull)	10.7	10.3 (96.3)	10.0* (93.5)	7.8** (72.9)
Day 11	27.4	26.1 (95.3)	25.6 (93.4)	18.0** (65.7)
Day 17	41.7	41.2 (98.8)	38.9* (93.3)	31.3** (75.1)
Day 21	53.9	52.1 (96.7)	50.4 (93.5)	41.3** (76.6)
Females				
Day 0	6.2	6.1 (98.4)	5.9* (95.2)	5.7** (91.9)
Day 4 (Pre-cull)	10.3	9.7 (94.2)	9.4** (91.3)	7.5** (72.8)
Day 4 (Post-cull)	10.3	9.7* (94.2)	9.4** (91.3)	7.5** (72.8)
Day 11	26.3	24.9 (94.7)	24.3* (92.4)	17.4** (66.2)
Day 17	40.3	39.2 (97.3)	36.7** (91.1)	29.5** (73.2)
Day 21	51.6	49.4 (95.7)	47.8* (92.6)	38.5** (74.6)

a Extracted from Table 9 (pages 73 and 74) of the study report.
b Percentage of control value
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Table 4b: Mean Postweaning Offspring Body Weight (g)[a]

	Fipronil Treatment			
	0	0.5	10	200
Males				
Week 1[b]	175.6	176.3 (100)[c]	167.1 (95.2)	145.8 (83.0)
Week 2	236.0	237.2 (100.5)	227.7 (96.5)	201.8 (85.5)
Week 3	296.3	295.2 (99.6)	285.9 (96.5)	256.4 (86.5)
Week 4	344.9	342.9 (99.4)	330.9 (95.9)	300.4* (87.1)
Week 5	369.5	361.4 (97.8)	358.6 (97.1)	317.0* (85.8)
Week 6	367.8	373.1 (100.4)	374.0 (101.7)	368.9 (100.3)
Females				
Week 1	141.3	139.6 (98.8)	134.3 (95.0)	120.0 (84.9)
Week 2	173.9	172.4 (99.1)	168.3 (96.8)	151.9 (87.3)
Week 3	200.1	198.8 (99.4)	192.8 (96.4)	177.2* (88.6)
Week 4	218.5	216.6 (99.1)	210.1 (96.2)	197.8* (90.5)
Week 5	232.9	226.5 (97.8)	220.5 (94.7)	203.3** (87.3)
Week 6	234.1	227.3 (97.1)	221.2 (94.5)	214.0 (91.4)

a Extracted from Table 9 (pages 75 and 76) of the study report.
b First week after all litters were weaned. Offspring ages were approximately 4-7 weeks at this time. This is the time period of sexual maturation.
c Percent of control in parenthesis.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

2. Pup Survival (see Table 5) - There was a statistically significant reduction in pup survival for the 200 ppm group on Lactation Day 4. The pup viability index[2] for Lactation Days 0-4 was 98.9% for control compared to 75.5% for the 200 ppm group. The pup weaning index[3] for the 200 ppm group was reduced, but the difference was not statistically significant because of the incorrect manner in which these values were calculated. Statistical analysis of corrected weaning indices was not performed. The pup viability and pup weaning indices for the 0.5 and 10 ppm groups were comparable to the control group.

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[2] Pup viability index = $\frac{\text{total pups alive (Day 4 precull)}}{\text{total pups born alive}} \times 100$

[3] Pup weaning index = $\frac{\text{total pups alive on Day 21}}{\text{total pups alive on Day 4 (post-cull)}} \times 100$

Litter survival (presence of at least one viable pup at weaning [Lactation Day 21]) was reduced in the 200 ppm group. All control litters (27 of 27 litters) survived until weaning, while only 85% (23 of 27 litters) of the 200 ppm group litters survived. All of the pups in the litters which did not survive died by Lactation Day 4 (although the death of individual pups at this dose level did occur after Day 4). Litter survival was not affected by 0.5 and 10 ppm fipronil treatment. Both treatment levels had 29 litters which survived until weaning.

Table 5: Pup Survival[a]

Observation	Treatment Level (ppm)			
	0	0.5	10	200
Mean Viability Index	98.9	95.7	98.3	75.5**
Mean Weaning Index[b]	86.9	86.8	87.1	81.9
Corrected Weaning Index[c]	99.5	99.5	99.5	85.1
Number of Pup Deaths[d,e]				
Days 0-4	4	19	8	105
Days 4-11	0	1	1	26
Days 11-21	1	0	0	0
Days 0-21	5	20	9	131

a Data extracted from Table 10 (page 78) of the study report.
b Pups sacrificed for brain weight/neuropathology evaluations on Day 11 were not accounted for in calculating the Mean Weaning Index. In general, for a litter with 8 pups on Lactation Day 4 (post-cull), which was reduced to 7 pups on Day 11, an index of 87.5% represents weaning of the entire litter.
c Calculated by the reviewer using the following calculation: number of pups alive at Day 21 X 100/ number of pups alive at Day 4 (post-cull) - number of pups sacrificed at Day 11.
d Includes found dead and missing pups; does not include pups culled at Day 4 or those selected for histopathology on Day 11.
e Extracted from pages 179-186 of the study report.
** Statistically different from controls, $p<0.01$

3. Dead Pup Observations - There was no evidence of a treatment-related effect on the dead pups. One male pup from a 200 ppm group litter found dead on Lactation Day 6 was observed to have a swollen, fluid-filled head and upper dorsal region. On gross examination, there was a red discoloration of the surface of the cranium and a separation of the frontal and parietal bones. The brain appeared to be

within normal limits.

4. Developmental Landmark Data - The study report summary (pages 40-41) states there were delays in lower incisor eruption and sexual development in the 200 ppm group males and females and in sexual development for the 10 ppm group males. However, the preface before Table 12 (pages 80-81) states there were statistically significant increases in the mean day of achieving pinna detachment and upper and lower incisor eruption noted for the 200 ppm group. For the sexual development landmarks, it states that females in the low and high dose groups had slower development relative to the control, but results of statistical tests are not discussed. The preface does state that there were statistical differences for the 10 and 200 ppm group males for day of achieving preputial separation. Table 12, which presents the data for these parameters, does not include statistical results. Vaginal patency and preputial separation were delayed for approximately 1.5 days and 4.8 days, respectively, for the 200 ppm group. The day of achieving preputial separation for the 10 ppm group males was 45.4 days vs. 44.0 days for the control group. The study report notes that in one previous study at this facility, the mean day of achieving separation was 45.0 with individual litter values ranging from 41.8 to 49.7 days. **Additional historical control data were faxed on May 9, 1997 and are attached to the DER in Appendix 1.** Huntingdon Life Sciences has conducted an additional developmental neurotoxicity study since performing this study. In the additional study, the mean day of achieving preputial separation was 44.9; the first and last days were 41 and 57, respectively. For females, the mean day for achieving vaginal opening was 31.9; the first and last days were 30 and 38, respectively.

The study report states there were statistically significant increases in the mean day of achieving pinna detachment, incisor eruption and sexual development which were less than one-half day or less. The study author did not consider these findings to be biologically significant since there is a normal variation arising from the inability to establish the exact time of birth within 12 hours. The EPA reviewer does not agree with this conclusion. Variation arising from establishing the exact time of birth would be seen across all dose groups, not just the high dose. In addition, the sexual maturation data should have been analyzed as postcoital (not postnatal) day of attaining completion, and the value should have been analyzed against the covariate of body weight, which is generally highly correlated to sexual maturation. **Revised tables for the developmental landmarks were faxed from the registrant on April 30, 1997 and are attached to the DER in Appendix 2.** The statistically

significant differences noted for the 200 ppm group were increased mean day to criteria for pinna detachment, upper and lower incisor eruption, vaginal patency and preputial separation. The mean day of achieving preputial separation for the 10 ppm group males was increased. Statistically significant changes in the 0.5 ppm group were not considered biologically significant. The revised data are presented in Table 6.

Table 6: Developmental Landmark Data[a]

Observation (Mean Day to Criteria)	Treatment Levels (ppm)			
	0	0.5	10	200
Pinna Detachment	2.5	2.6@@	2.5	3.0**
Upper Incisor Eruption	10.3	10.5@@	10.4	10.9**
Lower Incisor Eruption	11.4	11.6@@	11.4	12.1**
Eye Opening	13.9	13.9	13.8	14.0
Vaginal Patency	31.4	32.0^{0}	31.6	32.9^{00}
Preputial Separation	44.0	44.7	45.4^{00}	48.8^{00}

a Data extracted from revised Table 12 (page 82) of the study report.
@@ $p<0.01$ by logit regression
^{00} $p<0.01$ by Coxi regression
^{0} $p<0.065$ by Coxi regression

6. Mean Auditory Startle Data - **Revised statistical tables were also submitted for this parameter; see attached Table 13 in Appendix 2.** On Postnatal Day 22, there was a statistically significant decrease ($p<0.01$) in the maximum voltage response for males and females in the 200 ppm group at all five intervals. The effect was noted for all five blocks. There was no significant difference in the time to maximum response or average response for the 200 ppm group. There were no differences for the 0.5 and 10 ppm groups.

On Postnatal Day 60, the first three blocks of trials were performed without prepulse stimulus in order to assess habituation. There were no differences among control and treated groups in the maximum response, time to maximum response or average response.

7. Mean Motor Activity Data - **Revised statistical tables were also submitted for this parameter; see attached Table 14 in Appendix 2.** There were statistically significant ($p<0.01$) increases in mean motor activity counts for females in the 10 and 200 ppm groups on Postnatal Day 17. The study report states that this was not considered to be a

treatment-related effect but was attributed to motor activity in the female control group that was lower than expected based on comparison to the responses on Days 22 and 60. Nevertheless, the effect in females on Day 17 appears to be dose-related; control activity levels for both males and females on that day are comparable. Therefore, this increase in female motor activity on postnatal Day 17 is judged by the EPA reviewer to be treatment-related.

8. Swimming Development - **Revised statistical tables were also submitted for this parameter; see attached Table 15 in Appendix 2**. There was a slight delay in swimming development, as determined by direction and angle evaluations, for the 200 ppm group. Direction evaluations describe purposeful movement. As development proceeds, pups are able to stay afloat, swim in a circle and then swim in a straight line. Direction scores on Day 6 were reduced for the males and females in the 200 ppm group, although only the males were statistically significant ($p<0.01$). On this day, the incidence of control male and female pups unable to stay afloat (direction score of 0) was 11.5% and 7.7%, respectively. The incidence in the 200 ppm group males and females was 50.0% and 34.8%, respectively. The incidence of male and female pups which could swim in a straight line (score of 3) for the control group was 50.0% and 61.5%, respectively. The incidence for males and females in the 200 ppm group was 27.3% and 43.3%, respectively. The differences between this treated group and the control became less pronounced so that on Day 14, 95% of the 200 ppm group were swimming in a straight line as compared to 96% of control males and females.

Angle evaluations describe the position of the pup's head in the water. As development proceeds, the pup is able to hold more of its head out of the water. The group mean angle measurements were reduced for the 200 ppm group when compared to the control, although only the deficit for females was statistically significant according to the preface before Table 15. However, revised statistical tables do not indicate a statistical difference for females. On Days 6 and 8, a greater percentage of male and female pups in the 200 ppm group had their head submerged (score of 0), as compared to the controls. By Day 14, 92.3% of the control males and females had the top of the head, ears and nose above the surface (score of 3), while only 36.8% of males and 38.1% of females in the 200 ppm group were able to perform at this level.

Group mean paddling scores for males and females in the 200 ppm group were comparable to the control scores.

Group mean direction, paddling and angle scores for the 0.5

and 10 ppm groups were comparable to the control group.

8. Learning and Memory - Water "Y" maze time trials were performed on Postnatal Days 24, 25, 30, 60, 61 and 65. The study report states that the only statistically significant difference in times was observed for Trial #5 and #6 on Postnatal Day 24 for females in the 200 ppm group. The other trials for this group were comparable to the control group. The study report states that it is common for pups to demonstrate increases in trial times for the last trials in a phase. This is due to the fact that pups prefer to play in the water, rather than making attempts to find the correct arm of the maze. However, since this behavior only occurred at a significant level in the high-dose female pups and not in the controls, this was considered to be a treatment-related effect by the EPA reviewer. It is also noted that the time for 24 day-old male pups was increased substantially for trial 5 as well, although statistical significance was not found (high standard deviation).

Table 15, which contains summary data water "Y" maze time trial results from the original study report, is attached to the DER in Appendix 3.

C. POSTMORTEM EXAMINATIONS

1. Brain Weight Data (see Tables 7a and 7b) - Statistically significant decreases in absolute brain weights, relative to control values, were found on Postnatal Days 11 and 60 for the 200 ppm group males and females. Although, terminal body weights were also decreased for this group on these days, a biologically significant delay in brain development is demonstrated by these data. On Day 11, the relative brain weight for both sexes was significantly increased in comparison to the controls. On Day 60, the values for the control and 200 ppm groups were comparable. The group mean absolute and relative brain weights for the 0.5 and 10 ppm group were comparable to the control group.

Table 7a: Mean Male Pup Brain Weights (g)[a]

	Fipronil Treatment Level (ppm)			
	0	0.5	10	200
Day 11				
Terminal Body Weight	28.6	26.5	26.5	16.8** (-41%)[b]
Absolute Brain Weight	1.0496	1.0116	1.0254	0.8356** (-20%)
Relative Brain Weight	3.69	3.84	3.90	5.12** (+39%)
Day 60				
Terminal Body Weight	372.7	357.6	356.0	327.3** (-12%)
Absolute Brain Weight	1.9388	1.9600	1.9018	1.8039** (-7%)
Relative Brain Weight	0.52	0.55	0.54	0.55 (+6%)

a Extracted from Table 16 (page 97) of the study report.
b Change from control value
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

Table 7b: Mean Female Pup Brain Weights (g)[a]

	Fipronil Treatment Level (ppm)			
	0	0.5	10	200
Day 11				
Terminal Body Weight	25.9	23.8	24.7	19.3** (-25%)
Absolute Brain Weight	1.0297	0.9652	1.0103	0.9155* (-11%)
Relative Brain Weight	4.00	4.14	4.13	4.81** (+20%)
Day 60				
Terminal Body Weight	228.8	233.9	226.0	199.8** (-13%)
Absolute Brain Weight	1.8337	1.8111	1.8007	1.7023** (-7%)
Relative Brain Weight	0.81	0.78	0.80	0.86 (+6%)

a Extracted from Table 16 (page 98) of the study report.
b Change from control group.
* Statistically different from controls, $p<0.05$
** Statistically different from controls, $p<0.01$

2. Gross Postmortem Examinations of Maternal Females - There was no evidence of a treatment-related effect on the gross postmortem findings for the females sacrificed after the last litter was weaned.

3. Gross Postmortem Examinations of Pups - There was no evidence of a treatment-related effect on the gross postmortem findings for pups sacrificed on Days 11 and 60 or

at the termination of the study.

4. Histopathological Examinations - There were no histopathological examinations of tissues from the maternal females. There were no microscopic morphologic abnormalities in the brains of the control and 200 ppm group pups sacrificed on Day 11. Minimally dilated ventricles were seen in four pups from the control and five pups from the 200 ppm groups; morphometric measurement of the brain was apparently not performed.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study report concluded that the No Observed Effect Level (NOEL) was 0.5 ppm (0.05 mg/kg/day). The Lowest Observed Effect Level (LOEL) was 10 ppm, based on reduced pup weights. The treatment-related findings at 200 ppm included reduced food consumption, reduced body weight gains for maternal females, reduced pup survival, delays in incisor eruption, sexual development and swimming development and decreased maximum response voltage in auditory startle evaluations.

The investigator concluded that developmental neurotoxicity was only noted at concentrations where substantial maternal toxicity was observed, i.e. 200 ppm. The NOEL for developmental neurotoxicity was 10 ppm (0.9 mg/kg/day).

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY:

There was no evidence of a treatment-related effect on maternal survival or clinical signs of toxicity. Two females in the 200 ppm group died during lactation. There were no clinical signs of toxicity in these animals prior to death. On necropsy, discolored lungs were the only observation. The deaths were not attributed to fipronil. There were no clinical signs of toxicity, except for alopecia (general and extremities/snout), which was increased in the 200 ppm group.

Mean body weight for the 200 ppm group were reduced 15.5%, 10.0% and 8.6% in comparison to the controls on Gestation Days 10, 15 and 20, respectively. A statistically significant body weight loss was observed for Gestation Day interval 6-10, but mean body weight gain was significantly increased for interval 10-15. Statistically significant reductions in mean body weight were seen in the 200 ppm group on Lactation Days 0 and 4. Mean body weight gain was

statistically increased on Lactation Days 4-11.

A statistically significant reduction in group mean food consumption was noted in the 200 ppm group for Gestation Days 6 to 10 but were comparable to the controls for other intervals. The severe decrease in food consumption (50% of control) immediately following the introduction of fipronil into the diet suggests a palatability problem.

The data indicate that animals did adapt to the test material and the 200 ppm group was able to recover from the decreased food consumption. However, the effects on body weight extended well beyond when the animals were consuming normal amounts of food.

Pregnancy rate and gestation length for treated animals were comparable to the control group.

There was no evidence of a treatment-related effect on gross necropsy findings.

2. DEVELOPMENTAL TOXICITY/NEUROTOXICITY:

Litter size was not affected by fipronil treatment. There was a significantly increased number of stillborn pups in the 200 ppm group (0.1 for the control vs. 1.1 for the 200 ppm group). However, since the total number of pups born in the 200 ppm group was greater than the control group, the increase in stillborn pups did not result in a decrease in the mean number of pups born alive. The live birth index was decreased for the 200 ppm group, but the difference was not statistically significant. Pup sex distribution was not affected.

Group mean pup weights were reduced for the 10 and 200 ppm groups. During lactation, there was a statistically significant decrease for both males and females at 200 ppm at all recorded intervals and for various periods post-weaning. At 10 ppm, group mean weights were significantly reduced for females at all recorded intervals and for males on Days 4 and 17. Post-weaning weights were not affected.

Pup survival was affected at 200 ppm. The pup viability index (survival from Postnatal Days 0-4) for the 200 ppm group was significantly decreased (98.9% for control vs. 75.5% for the 200 ppm group). Litter survival was reduced in the 200 ppm group. All of the control group litters survived until weaning, while only 85% of the 200 ppm group survived. All of the pups in the lost litters died by Day 4 of lactation. The weaning index (survival from Postnatal Days 4-21) was decreased for this group, but the difference was not statistically significant.

Statistically significant increases in the mean day of achieving pinna detachment, upper and lower incisor eruption, vaginal patency and preputial separation were noted for the 200 ppm group. Vaginal patency and preputial separation were delayed for approximately 1.5 days and 4.5 days, respectively. There was a statistically significant increase in the mean day of achieving preputial separation for the 10 ppm group males (45.4 days for the 10 ppm group vs. 44.0 days for the control group). The study report notes that in a previous study at this facility, the mean day of achieving separation was 45.0 with individual values ranging from 41.8 to 49.7 days. In the additional historical control data, the mean day of achieving this criterion was 44.9; the first and last day were 41 and 57, respectively. Examination of the individual data for the day of achievement in this fipronil study shows there was a range of 42 to 57 days. Eight animals had values of 50 or more; the median day was 45. It appears that this was a treatment-related delay. Therefore, it is concluded that fipronil had an effect on sexual and non-sexual development parameters in male and females at 200 ppm and on sexual parameters (preputial separation) in males at 10 ppm.

Auditory startle testing on Postnatal Day 22 demonstrated a statistically significant decrease in the maximum voltage response for males and females in the 200 ppm group at all five intervals. The effect was noted for all five blocks. There was no significant difference in the time to maximum response or average response for the 200 ppm group. There were no changes in this parameter on Postnatal Day 60.

Motor activity testing on Postnatal Day 17 showed statistically significant increases in motor activity counts for females in the 10 and 200 ppm groups. The study report states that this was not considered to be a treatment-related effect but was attributed to motor activity in the female control group that was lower than expected based on comparison to the responses on Days 22 and 60. Nevertheless, the effect in females on Day 17 appears to be dose-related; control activity values levels for both males and females on that day are comparable. Therefore, this increase in female motor activity on postnatal Day 17 is judged by the EPA reviewer to be treatment-related.

Swimming direction scores on Day 6 were reduced for the males and females in the 200 ppm group, although only the males were statistically significant. On this day, the incidence of control male and female pups unable to stay afloat (direction score of 0) was 11.5% and 7.7%, respectively. The incidence in the 200 ppm group males and females was 50.0% and 34.8%, respectively. The incidence of male and female pups which could swim in a straight line

(score of 3) for the control group was 50.0% and 61.5%, respectively. The incidence for males and females in the 200 ppm group was 27.3% and 43.3%, respectively. The differences between this treated group and the control became less pronounced so that on Day 14, 95% of the 200 ppm group were swimming in a straight line as compared to 96% of control males and females.

The swimming group mean angle measurements were reduced for the 200 ppm group when compared to the control, although the deficit was not statistically significant (according to the revised tables with statistical notation). On Days 6 and 8, a greater percentage of male and female pups in the 200 ppm group had their head submerged (score of 0), as compared to the controls. By Day 14, 92.3% of the control males and females had the top of the head, ears and nose above the surface (score of 3), while only 36.8% of males and 38.1% of females in the 200 ppm group were able to perform at this level.

Water "Y" maze time trials for learning and memory showed statistically significant differences in times for Trials #5 and #6 on Postnatal Day 24 for females in the 200 ppm group. The other trials for this group were comparable to the control group. The study report states that it is common for pups to demonstrate increases in trial times for the last trials in a phase. This is due to the fact that pups prefer to play in the water, rather than making attempts to find the correct arm of the maze. However, since this behavior only occurred at a significance level in the high-dose female pups and not in the controls, this was considered to be a treatment-related effect by the EPA reviewer. It is also noted that the time for 24 day-old male pups was increased substantially for trial 5 as well, although statistical significance was not found (high standard deviation).

Statistically significant decreases in absolute brain weights, relative to control values, were found on Postnatal Days 11 and 60 for the 200 ppm group males and females. Although, terminal body weights were also decreased for this group on these days, a biologically significant delay in brain development is demonstrated by these data. On Day 11, the relative brain weights for both sexes were significantly increased in comparison to the controls. On Day 60, the relative brain weight values for the control and 200 ppm groups were comparable.

There were no treatment-related macroscopic postmortem effects seen in the maternal females sacrificed after weaning. There was no evidence of a treatment-related effect on the gross macroscopic or microscopic examinations

(including the central and peripheral nervous systems) of the pups sacrificed on Postnatal Days 11 and 60, although morphometric evaluations of pup brains were not performed.

C. STUDY DEFICIENCIES

1. The study deviates from the Developmental Neurotoxicity Study Guidelines (OPPTS 870.6300) in the following areas:

a. The intermediate dose group was not equally spaced between the low and high dose groups.

b. Morphometric studies to assess the structural development of the brain were not included with the neuropathological examinations done on Postnatal Days 11 and 60, although visual evaluations were done.

c. The olfactory bulbs, hypothalamus and thalamus were not examined.

d. The Guidelines state that gross examination of the dams should be made at least once daily by trained technicians blinded to the treatment group. Observations should include assessments of signs of autonomic function and description, incidence and severity of abnormal movements, gait abnormalities and behavior abnormalities. According to the study report, physical examinations were done on Days 0 and 6-21 of gestation; females with litters were examined on Days 1-10, 14 and 21 of lactation. In addition, examination of the raw data does not indicate that detailed examinations, as described in the Guidelines, were completed.

e. Positive control data from the laboratory conducting the study were not submitted for the sexual developmental parameters. In addition, the auditory startle response data measured mean response with stimulus, mean response with no stimulus and the difference. These measurements differ from the endpoints (maximum response, time to maximum response and average response) determined in the present study. In addition, motor activity evaluations were done on Days 13, 17, 22 and 42, as compared to Days 13, 17, 22 and 60 in the present study.

2. The statistical analyses which are discussed in the prefaces before data tables are incomplete and difficult to understand. For example, "The Summary of Swimming Development Evaluations and Water "Y" Maze Time Trial Results Preface" does not discuss the Water "Y" results. In addition, the tables, except Table 15 (page 94), do not indicate which results are statistically significant nor were separate tables or lists of "p" values provided to allow the EPA reviewer to

evaluate the statistical analyses. Revised tables with statistical notations were submitted for developmental landmarks (Table 12), Day 22 Mean Auditory Startle (Table 13), Day 17 Mean Motor Activity (Table 14) and Summary of Swimming Development Evaluations (Table 15).

D. NEUROTOXICITY FINDINGS IN OTHER FIPRONIL STUDIES

Neurotoxicity has been a consistent finding in subchronic and chronic studies in multiple species. In this developmental neurotoxicity study, maternal females were treated from Gestation Day 6 to Lactation Day 10, approximately 26 days. There were no treatment-related clinical signs of toxicity, even at the 200 ppm (15 mg/kg/day) dose.

In the combined chronic toxicity/carcinogenicity study (MRID # 42918648), rats treated at doses as low as 1.5 (males: 0.059 mg/kg/day; females: 0.078 mg/kg/day) and 30 ppm (males: 1.27 mg/kg/day; females: 1.61 mg/kg/day) were observed to have seizures. Other clinical signs of neurotoxicity included irritability, overactivity, vocalization, salivation, aggressive behavior and grinding of the teeth.

In the carcinogenicity study in mice (MRID # 42918649), convulsions were observed in the 60 ppm (approx. 6 mg/kg/day) group males during Week 2 of the study.

In the subchronic study in dogs (MRID # 42918642), one male and three females in the 10.0 mg/kg/day group were euthanized during the second week of treatment due to poor condition. Clinical signs in these animals attributed to neurotoxicity included subdued behavior, excessive salivation, hindlimb extension, convulsions, disorientation and absent menace reaction.

In the chronic toxicity study in dogs (MRID 42918645), signs of neurotoxicity were observed at 2 and 5 mg/kg/day in Week 2 of treatment.

There were other studies at comparable doses and durations to the present developmental neurotoxicity study in which neurotoxicity was not observed. In the rat developmental study (MRID # 42977903), animals were dosed at 0, 1, 4 or 20 mg/kg/day. The maternal NOEL was 4 mg/kg/day; the LOEL was 20 mg/kg/day, based on reduced body weight gain, increased water consumption, reduced food consumption and reduced food efficiency. The developmental NOEL was 20 mg/kg/day or higher; the LOEL was greater than 20 mg/kg/day. In the rabbit developmental study (MRID # 42918646), animals were dosed at 0, 0.1, 0.2, 0.5 or 1.0 mg/kg/day. The maternal NOEL was less than 0.1 mg/kg/day; the maternal LOEL was equal to or less than 0.1 mg/kg/day, based on reduced body weight gain, reduced food consumption and efficiency. The developmental NOEL was equal to or greater than 1.0 mg/kg/day; the

In the reproduction study (MRID # 42918647), fipronil was administered at doses of 0, 3, 30 or 300 ppm (males: 0, 0.25, 2.54 or 26.03 mg/kg/day; females: 0, 0.27, 2.74 or 28.40 mg/kg/day). The parental (systemic) LOEL was 30 ppm based on increased weight of the thyroid glands and liver in males and females, decreased weight of the pituitary in females and increased incidence of thyroid follicular epithelial hypertrophy in females. The NOEL was 3 ppm. The LOEL for reproductive toxicity was 300 ppm based on clinical signs of toxicity in the F_1 and F_2 offspring, decreased litter size in the F_1 and F_2 litters, decreased percentage of F_1 parental animals mating, decrease in fertility index in F_1 parental animals, decreased post-implantation survival and offspring postnatal survivability in F_2 litter and delay in physical development in F_1 and F_2 offspring. (There was a statistically significant delay in tooth eruption in F_1A offspring of the 300 ppm group. In the F_2 generation, pinna unfolding was delayed, but not significantly, for the 300 ppm group.) The NOEL was 30 ppm.

The requirement of a developmental neurotoxicity study was made at the July 21, 1994 RfD Committee Meeting. The memo of that meeting indicates that there was no evidence that fipronil was associated with significant reproductive or developmental toxicity in the studies reviewed at the meeting. The Committee recommended a developmental neurotoxicity study be conducted due to the consistent evidence of neurotoxicity in adult animals and the mechanism of action of the chemical, i.e. GABA inhibitor.

In the acute neurotoxicity study (MRID # 42918635), rats were administered a single dose of 0, 0.5, 5.0 or 50 mg/kg of fipronil. The LOEL was 5.0 mg/kg based on decreased hind leg splay at the 7 hours post-treatment. The NOEL was 0.5 mg/kg.

In the subchronic neurotoxicity study (MRID # 43291703), rats were administered 0, 0.5, 5.0 or 150 ppm (males: 0, 0.029, 0.301 or 8.89 mg/kg/day; females: 0, 0.0354, 0.351 or 10.8 mg/kg/day) of fipronil. The LOEL was 150 ppm based on functional observation battery findings [increased incidence of exaggerated tail pinch response (males only), increased incidence of exaggerated startle response in manipulative observation and increased forelimb grip strength (females only).] The NOEL was 5.0 ppm.