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Toxicology Branch II (7509C)

Marion Copley Date: 4/28/98

Stephen C. Dapson Date: 4/29/98

DATA EVALUATION RECORD - supplemental
Original DER is in TXR # 012290

012624

STUDY TYPE: Developmental Neurotoxicity Study - rat; OPPTS 870.6300

DP BARCODE: D243904
P.C. CODE: 129121

SUBMISSION CODE: S538688
TOX CHEMICAL NO.: none

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

SYNONYMS: MB 46030

CITATION: Mandella, R. C., D.E. Rodwell (1998) Historical Control Data in Support of Study No. 93-4508: 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, 27-FEB-1998, MRID 44501102. Unpublished

Bieler, G.S. (1998) Analysis of Mean Pup Body Weights for the Fipronil Neurotoxicology Study. Research Triangle Institute, RTP, NC, RTI Project Number 6161-3, 26-FEB-1998, MRID 44501103. Unpublished

original DER 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 Unpublished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 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. *At 10 ppm* there was a statistical decrease in pup weight gain and delayed preputial separation. *At 0.5 ppm* there were no treatment related effects. **The developmental toxicity LOEL is 10 ppm (0.90 mg/kg/day), based on a marginal but statistically significant decrease in group mean pup weights during lactation, and significant increase in time of preputial separation in males. The NOEL for developmental toxicity is 0.5 ppm (0.05 mg/kg/day).**

At 200 ppm 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. 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. **The developmental neurotoxicity LOEL is**

200 ppm (15 mg/kg/day) based on: decreased auditory startle response; reduced swimming direction scores, group mean angle measurements and water "Y" maze times trails; and decreased absolute brain weights. The NOEL for developmental neurotoxicity is 10 ppm (0.90 mg/kg/day).

It is noted that developmental toxicity occurred at a dose lower than the maternal toxicity NOEL in this study. However, the HED Hazard ID Assessment Review Committee (meeting dated 22-APR-1998) ***did not consider this to indicate increased susceptibility to infants and children (see discussion section below and the HIARC document for details)***

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.

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Rhone-Poulenc has submitted additional data to OPP regarding a developmental neurotoxicity study with fipronil. The intent of this data is to support the Registrant's claim that the NOELs for Developmental Toxicity and Neurotoxicity are not lower than that for Maternal Toxicity.

The **Maternal Toxicity LOEL** in the original DER (TXR # 012290) of 200 ppm (15 mg/kg/day), is based on decreased body weight, body weight gain and food consumption. The Maternal NOEL was established at 10 ppm (0.90 mg/kg/day). The **Developmental LOEL** in the original DER was established at 10 ppm (0.90 mg/kg/day), based on statistically significant decreases in group mean pup weights during lactation and significant increases in time of preputial separation in males. The **Developmental Neurotoxicity LOEL** was established at 10 ppm (0.09 mg/kg/day) based on a significant increase in mean motor activity counts in females on Postnatal Day 17. The Developmental and Developmental Neurotoxicity NOELs were established at 0.5 ppm (0.05 mg/kg/day).

The Registrant does not address the Maternal Toxicity NOEL and LOEL. They do present data and arguments to support their claim that for both the **Developmental** and **Developmental Neurotoxicity** endpoints, the NOELs and LOELs are 10 ppm (0.90 mg/kg/day) and 200 ppm (15 mg/kg/day), respectively, the same as those for Maternal Toxicity. *They argue that there is no support for increased developmental susceptibility.*

This evaluation will address the toxicity endpoints of concern for Developmental and Developmental Neurotoxicity separately, first presenting the conclusion in the original DER, then Registrant's rebuttal followed by HED's evaluation of the rebuttal.

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1) mean pup weights:

- The **original DER** states that there is a statistically significant decrease in group mean pup weights during lactation (table 1 is taken from table 4a of the original DER) at 10 ppm.
- **The Registrant** presented 2 arguments. The first consisted of historical control data for 12 multigeneration studies between 1989 and 1995 (the present study was completed in 1995). The time period evaluated in the reproduction study was the same as that evaluated in the developmental neurotoxicity study (days 0 - 21 post-partum). They presented it in the form of graphs (see attached figure 1 copied from Figure 1, page 8, MRID 44501102 of the submission) and as a table. The graph demonstrates that the weights are very close to those for the means of the 12 historical control studies. When the table values (low and high range) for days 4 (pre-cull) and 21 are compared (see table 2) to the 10 ppm value, It is evident that the 10 ppm values are well within the historical control range. The second argument refers to the observation that in the fipronil multigeneration study, at 30 ppm (a dose higher that the 10 PPM in this study) there was no decrease in pup weight for either generation.
- **HED interpretation:** The HIARC disagrees with the Registrant's discussion that decreased pup weight gains should not be attributed to treatment at the mid dose. The HIARC, following careful review of the Registrant's rationale and data, concluded that the decrease in

pup weights is treatment-related because: the decrease in pup weights at this dose showed statistical significance when compared to controls; 2) the decrease was seen in both males and female pups; 3) the decrease was consistently observed at Days 4 (pre- and post cull), Day 11 (not statistically significant in males), 17 and 21; 4) the decrease occurred in a dose-dependent manner; and 5) the decrease in pup weight is correlated with increase in preputial separation time. The HIARC did however, concur with the Registrant that the values (decreases in pup weight gains) at 10 ppm were 1) marginal; 2) within the historical control range; 3) the percent total pup weight gain as compared to day 0 post partum weights is not increased until the high dose (table 3), and that 3) similar decreases in pup weights were not observed in the two-generation reproduction study at a slightly higher dose (30 ppm). However, the biological significance and/or relevance of these effects can not be fully discounted in this study since these effects were: 1) statistically significant in both males and females; 2) more pronounced at 200 ppm, 3) in the presence of delayed preputial separation. Therefore the NOEL for pup weight gain is 0.5 ppm and the LOEL is 10 ppm.

Table 1: 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 2: Comparison of the 10 PPM Mean Pup Weights (g) to the Range of 12 Historical Control (HC) Studies

Post Partum day / Group[1]	0	4 (pre cull)	21
concurrent control	6.3	10.5	52.8
10 ppm	6.1	9.7	49.1
HC mean	6.1	9.3	48.2
HC low value	5.7	8.6	42.9
HC high value	6.4	10.5	54.1

1 All groups are presented as means of males and females since this is the only way the historical control data base was kept.
Data are extracted from Table 2, Page 12 of MRID 44501102.

Table 3: Percent Total Lactation Weight Gain as Compared to Day 0 Post Partum Weights[1]

Group[1] (ppm)	Males	Females
control	730	730
0.5	700	710
10	700	710
200	600	570

1 {(day 21 weight - day 0 weight) / day 0 weight} x 100 = %
Data are extracted from Table 2, Page 12 of MRID 44501102.
Calculated by reviewer.

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2) <u>increases in time of preputial separation</u>:

- The **original DER** states that there is a significant increase in time of preputial separation in males (day 44.0±2.5, 44.7±2.5, 45.4±2.9**, 48.8±3.3** for controls to 200 ppm) (** $p<0.01$ by Coxi regression) - data taken from table 12 of the original study. Only two historical control studies were available (a) mean = 45 (41.8-49.7); (b) mean = 44.9 (41 - 57)).
- **The Registrant** states that, while there is evidence of a slight delay at 10 ppm, the "effect must be considered of doubtful biological significance since the mean of the 10 ppm group is within half a standard deviation of the control group." The Registrant also included additional historical control data for 5 studies (A - E) conducted between 1993 and 1998 (see attached table 3 from page 15 of MRID 44501102). This table didn't include study b) above.
- **HED interpretation** -
  - The Committee felt that the inhalation historical control data (study D) were not appropriate for comparison since the route of exposure was so different. The remainder of the historical

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control data do not appear to support the Registrant's conclusion since:

1) the mean for time of preputial separation at 10 ppm is longer than all means for the available historical control studies (except for the inhalation study); and

2) the range maximum of 53.3 days in the 10 ppm group was outside all historical control ranges, except for the historical control study [mean = 44.9 (41 - 57)] mentioned in the original DER(b) that is not on the table.

- The HIARC acknowledged that the standard deviation overlaps for the controls and the 10 ppm dose were of some concern, but felt that this alone did not support the Registrant's conclusion.
- In addition, the HIARC considered the decreased body weight gain to be related to the increased preputial separation time.

Therefore, the HED HIARC concluded that these arguments do not conclusively support that increased time of preputial separation is unrelated to treatment.

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3) increase in mean motor activity counts:

- **Original DER** - 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
- The **Registrant states** that motor activity is not altered due to treatment at any dose. They use as support, 17 day historical control data from 3 studies (Attachment 3 - figure 2 and 3, page 9, 10 and Attachment 4 - table 4, page 16 of MRID 44501102). When figure 2 is examined it is evident that for all 5 minute interval values on day 17 the control is less than all 3 treated groups **AND** that there is no dose related response for the 3 treatment groups. The low and high dose values are similar, for the most part, and the mid dose group actually has the highest activity at all 5 minute intervals. In addition, in figure 3, it is evident that the concurrent control values are lower than all three control studies for all but one 5 minute interval. They also observe that the motor activity curves of the mid and high dose groups falls within the range of the controls. The Registrant concludes that: 1) since the concurrent historical control data is unusually low; 2) there is no dose related response; and 3) it is unlikely that day 17 females would be effected by treatment when there is no effect at any dose for females at days 13, 22 or 60 or at any time in males, there is no treatment related change in motor activity due to treatment with fipronil.
- **HED interpretation** - The HIARC concurred with the rationale provided by the Registrant and determined that the increases in motor activity observed at the mid- (10 ppm; 0.9 mg/kg/day) and high-(200 ppm; 15 mg/kg/day) are in fact due to variation and not treatment

based on the following factors: 1) lack of a dose response at the doses tested; 2) the values for the concurrent controls were lower than the historical control values; 3) the motor activity curves at the mid-and high-doses were within the range of the historical controls; and 4) the effect seen in females at Day 17 was an isolated incidence since similar increases were not seen in females at days 13, 22 or 60 or in males at any of the time intervals tested.

Based on this, the HIARC determined that for developmental neurotoxicity, the NOEL was 10 ppm (0.9 mg/kg/day); The LOEL is 200 ppm (15 mg/kg/day) based on decreased auditory startle response; reduced swimming direction scores, group mean angle measurements and water "Y" maze times trails; and decreased absolute brain weights.

Conclusions: The developmental neurotoxic NOEL and LOEL should be increased to 10 and 200 ppm since there was no treatment related increased in motor activity. Treatment related effects attributable to fipronil did occur at 200 ppm. The HED HIARC concluded that the developmental NOEL should remain at 0.5 ppm based on decreased pup weight gain (day 0 to weaning) and delayed preputial separation. All other conclusions in the original DER are considered accurate as stated. The executive summary above takes these new conclusions into consideration and **should supercede the executive summary presented in the DER in TXR 012290.**

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