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Chemical:	**Fipronil**
PC Code:	**129121**
HED File Code	**13000 Tox Reviews**
Memo Date:	**03/05/1996**
File ID:	**DPD213806**
Accession Number:	**412-01-0170**

HED Records Reference Center
05/22/2001

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAR - 5 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Fipronil: Waiver for the Chronic Neurotoxicity Study in Rats

PC Code: 129121 DP Barcode: 213806
Submission: S484677

FROM: Yiannakis M. Ioannou, Ph.D., Head
Review Section I, Toxicology Branch II
Health Effects Division (7509C)

Y. M. Ioannou 3/4/96

TO: Richard F. Griffin
Registration Section, RCAB
Health Effects Division (7509C)

THROUGH: Stephanie Irene, Ph.D., Acting Chief
Toxicology Branch II
Health Effects Division (7509C)

Stephanie Irene 3/5/96

Registrant: Rhone-Poulenc Ag Company, RTP, NC.

Action Requested: Review the protocol for a proposed chronic neurotoxicity study with Fipronil in rats

Recommendations: Based on reevaluation of all the available neurotoxicity data on Fipronil, it was determined that the required chronic neurotoxicity study in rats would not change the overall picture as to the neurotoxic potential of Fipronil. Thus, this requirement is now waived; consequently, a review of the submitted protocol is not warranted.

Background:

The Health Effects Division RFD/Peer Review Committee met on July 21, 1994 to discuss and evaluate all available toxicology data submitted in support of Fipronil registration and to assess the Reference Dose for this chemical. During this meeting, the Committee concluded that a "chronic neurotoxicity study in adult rats must be submitted." This requirement was based on neurotoxic effects (including seisures and other excitatory effects) observed in the acute neurotoxicity study and the chronic rat studies and the available information for potential inhibition of GABA receptors by Fipronil.

The Registrant, Rhone-Poulenc Ag Company, submitted for review by the Agency (April,1995) a protocol entitled: "52-Week Dietary Chronic Neurotoxicity study with Fipronil in Rats". Subsequent to the submission of the protocol a completed 90-day neurotoxicity study was submitted to the Agency for review (May,1995). The review of the chronic neurotoxicity protocol was delayed until the 90-day neurotoxicity study was reviewed so that the Agency will have a better picture of the neurotoxicity potential of Fipronil. Upon completion of the review of the 90-day neurotoxiciy study, HED scientists (W. Sette, R. Fricke, Y. M. Ioannou), based on the results of this study, and all the available data from previously submitted studies, decided to reconsider the requirement for the chronic neurotoxicity study in the rat.

Discussion/Conclusions

The original recommendations for a chronic neurotoxicity screening study of Fipronil arose from concerns for signs seen in chronic toxicity studies and from the lack of any repeated exposure studies with Fipronil tailored to screen for neurotoxic effects.

Since that recommendation, a subchronic neurotoxicity study (MRID 432917-03), performed according to the specifications contained in the EPA Guideline for Neurotoxicity Screening Studies has been submitted, reviewed and found to be acceptable (HED Doc. No. 011772).

The protocol submitted is for a screening neurotoxicity study in which the animals are exposed chronically, i.e., in this instance, for 12 months.

Given that it is already known that this material has specific action related to the nervous system, i.e., it inhibits GABA, a brain neurotransmitter, and the excitatory phenomena seen at high doses, it seems scientifically that the next step should be aimed at further characterization of those effects, that is, the neurochemical effects and their functional correlates.
Neurochemically, this would involve dose response functions for acute and repeated exposure relating exposure to GABA inhibition in

appropriate neural tissues. This seems a basic need, akin to the development and use of cholinesterase data for organophosphates and carbamates. Functionally, for convulsant related effects, this might well be pursued by electrophysiological means, and by neuropharmacological interactions with standard convulsants such as pentylenetetrazole. This is what might be viewed as a higher tier effect than the neurochemical testing.

In view of the availability of an acceptable subchronic neurotoxicity study according to EPA guidelines, the low RfD currently established for this chemical, and the fact that the proposed chronic study would only use screening methods in a longer duration study, it was decided that this chronic study be waived.

It is recommended that the registrant submit to the Agency all available neurochemical data on the potency of GABA inhibition following acute as well as repeated exposures to Fipronil.

Based on the decision to waive the requirement for the conduct of the chronic neurotoxicity study in rats, a review of the submitted protocol is not warranted at present.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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REVIEWER

JAN 30 1996

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

SUBJECT: **FIPRONIL** Subchronic Neurotoxicity Screening Battery - Rats
[OPPTS 870.6200, §82-7]

EPA ID NOs.: MRID No.: 432917-03
DP Barcode:: D214926
Submission No.: S476260
P.C. Code: 129121
TOX Chem No.: none

FROM: Robert F. Fricke, Ph.D. Robert F. Fricke 25 Jan 96
Toxicology Branch II, Section II
Health Effects Division (7509C)

TO: Rick Keigwin
Product Manager (10)
Registration Division (7505C)

THRU: K. Clark Swentzel K. Clark Swentzel 1/25/96
Toxicology Branch II, Head Section II
Health Effects Division (7509C)

and

Stephanie Irene, Ph.D. Stephanie F. Irene 1/26/96
Acting Branch Chief, Toxicology Branch II
Health Effects Division (7509C)

REGISTRANT: Rhône-Poulenc Ag Company, 2 T.W. Alexander Drive, Research Triangle Park, NC

CHEMICAL: M&B 46030, Fipronil (96.7%):

ACTION REQUESTED: Review subchronic neurotoxicity study in the rat.

EXECUTIVE SUMMARY: Driscoll, C.D. and Hurley, J.M. (15 September 1993), M&B 46030: Ninety-day dietary neurotoxicity study in Sprague-Dawley rats,

Bushy Run Research Center, Export, PA, Report No.: 92N1074, MRID No.: 43291703, Unpublished

In this subchronic neurotoxicity study, male and female Sprague-Dawley rats (15/sex/dose) were fed test diets containing M&B 46030 at 0 (basal diet), 0.5, 5.0, or 150 ppm (equivalent to 0, 0.0297, 0.301, or 8.89 mg/kg/day, males; 0, 0.0354, 0.351 or 10.8 mg/kg/day, females). Neurobehavioral screening, consisting of Functional Observational Battery and motor activity evaluations, was performed at pretreatment, and during Weeks 4, 9 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

With the exception of one low-dose female which was found dead on Day 16, all remaining animals survived to terminal sacrifice without the appearance of any treatment-related clinical signs.

Decreases in mean body weight, observed in high-dose males and females at Week 1 of treatment, were judged to be slight (6.5%, males; 6.9%, females). The decrease in body weight was accompanied by a concomitant decrease in food consumption, which would suggest a palatability problem, rather than a treatment-related effect.

FOB findings revealed minimal effects in high-dose animals at the Weeks 4, 9 and 13 evaluations. High-dose males had a decreased incidence of no urination and an increased incidence of exaggerated tail pinch response. High-dose males and females had an increased incidence of exaggerated startle responses in the manipulative observations. High-dose females had increased forelimb grip strength at Week 13. The mean body weights of treated males were significantly greater than the concurrent control values.

Necropsy findings did not reveal any treatment-related gross pathological or histopathological findings. Although histopathological lesions were observed, incidences were low and attributed by the study pathologist to animal variation and artifactual changes.

Based on the results (FOB findings) of this study, the LOEL was established at 150 ppm (8.89 mg/kg/day, males; 10.8 mg/kg/day, females); the NOEL was established at 5.0 ppm (0.301 mg/kg/day, males; 0.351 mg/kg/day, females).

This study is classified as **Core - Acceptable** and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.

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EPA Reviewer: Robert F. Fricke, Ph.D. Robert F. Fricke 25 Jan 96
Review Section II, Toxicology Branch II, HED (7509C)

EPA Secondary Reviewer: K. Clark Swentzel K. Clark Swentzel 1/25/96
Review Section II, Toxicology Branch II, HED (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Neurotoxicity Screening Battery - Rats [OPPTS 870.6200, §82-7]

DP BARCODE: D214926
P.C. CODE: 129121

SUBMISSION NO.: S476260
TOX CHEM NO.: none

TEST MATERIAL M&B 46030, Fipronil (96.7%): 5-Amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-(1,R,S)-(trifluoromethyl)sulfinyl-1H-pyyrazole-3-carbonitrile

CITATION: Driscoll, C.D. and Hurley, J.M. (15 September 1993), M&B 46030: Ninety-day dietary neurotoxicity study in Sprague-Dawley rats, Bushy Run Research Center, Export, PA, Report No.: 92N1074, MRID No.: 43291703, Unpublished

SPONSOR: Rhône-Poulenc Ag Company, 2 T.W. Alexander Drive, Research Triangle Park, NC

EXECUTIVE SUMMARY: In this subchronic neurotoxicity study, male and female Sprague-Dawley rats (15/sex/dose) were fed test diets containing M&B 46030 at 0 (basal diet), 0.5, 5.0, or 150 ppm (equivalent to 0, 0.0297, 0.301, or 8.89 mg/kg/day, males; 0, 0.0354, 0.351 or 10.8 mg/kg/day, females). Neurobehavioral screening, consisting of Functional Observational Battery and motor activity evaluations, was performed at pretreatment, and during Weeks 4, 9 and 13. At terminal sacrifice, six animals/sex/dose were anesthetized and perfusion fixed *in situ* for neuropathological evaluation.

With the exception of one low-dose female which was found dead on Day 16, all remaining animals survived to terminal sacrifice without the appearance of any treatment-related clinical signs.

Decreases in mean body weight, observed in high-dose males and females at Week 1 of treatment, were judged to be slight (6.5%, males; 6.9%, females). The decrease in body weight was accompanied by a concomitant decrease in food consumption, which would suggest a palatability problem, rather than a treatment-related effect.

I. MATERIALS

A. Test Material: M&B 46030, Fipronil
Description: White powder
Batch No: 78/GC/90
Purity: 96.7%
CAS No.: 120068-37-3

B. Test Animals
Species (Strain): Rat (Sprague-Dawley)
Age at Initiation: Approximately 8 weeks
Weight at Initiation: 251.2-279.9 g (males), 162.6-192.3 g (females)
Source: Harlan Sprague-Dawley, Indianapolis, IN
Housing: one/cage during study
Feed: Ground Purina Rodent Chow #5002, *ad libitum*
Water: Tap water, *ad libitum*
Environment: Temperature: 67-77°F; Humidity: 40-70%
Air changes: Not stated; Light/dark cycle: 12 hr/12 hr
Acclimation Period: 3 Weeks

II. STUDY DESIGN

A. In-Life Study Dates: 22 June 1992 to 23-25 September 1992

B. Animal Assignment: Animals were randomly assigned to study groups as presented in Table 1. Weight variations at initiation of the study were limited to ± 20% of the population mean for each sex.

Table 1: Study Design

Study Group	Dose Level (ppm)	Animals Assigned	
		Males	Females
Control	0	15	15
Low	0.5	15	15
Mid	5.0	15	15
High	150	15	15

C. Dose Selection Rationale: Dose selection was based on a chronic toxicity/carcinogenicity study (MRID No.: 429186-48, HED Doc. No.: 011086) in which rats were fed diets containing 0, 0.5, 1.5, 30, or 300 ppm M&B 46030. At 300 ppm, three males and two females died within the first week of treatment. Convulsions were observed in these animals prior to death. Two additional animals were found dead during Weeks 3

1. **Functional Observational Battery:** The following parameters were evaluated in 10 animals/sex/group by a trained observer at each test session:

CAGESIDE OBSERVATIONS	**MANIPULATIVE OBSERVATIONS**
Posture	Approach response
Convulsions	Startle response
Tremors	Tail pinch response
Palpebral closure	Pupil response
OPEN-FIELD OBSERVATIONS	Muscle tone
Reactivity to handling	Lacrimation
Convulsions	Salivation
Tremors	Exophthalmus
Vocalizations	Emaciation
Piloerection	Dehydration
Abnormal/stereotypic behavior	Fur appearance
Gait abnormalities	Crusts
Body position	Visual placing
Breathing pattern	**MEASUREMENTS/COUNTS**
Arousal	Body weight
Palpebral closure	Body temperature
Quantity of urine	Air righting
Number of fecal pellets	Hind/Forelimb grip strengths
No. of rears	Landing foot splay

2. **Motor Activity:** Following the FOB evaluations, 15 animals/sex/group were evaluated for motor activity using an automated, figure-eight shaped activity chamber. Animals were monitored individually during a 90-minute session, consisting of nine, 10-minute intervals. Motor activity of control animals was asymptotic during the last 20 minutes of the evaluation.

E. Neuropathology: During Week 14 of treatment, 10 animals/sex/group (11 in the high-dose female group) were anesthetized and perfused *in situ* with 10% neutral buffered formalin. Gross examination of the thoracic and peritoneal cavities were performed on all animals. Neuropathological examinations were performed on 6 animals/sex from the control and high-dose groups. Brains were weighed prior to sectioning. The following tissues were examined:

GROSS LESIONS
BRAIN: Cerebral cortex, cerebellar cortex, medulla/pons
SPINAL CORD: Cervical, thoracic & lumbar regions
CENTRAL NERVOUS SYSTEM: Dorsal root ganglia, dorsal & ventral nerve roots, & gasserian ganglion
PERIPHERAL NERVES: Sciatic, tibial, sural, & peroneal nerves
THYROIDS
LIVER
TAIL (animal identification only)

Table 3: Mean Body Weight (% Change from Control) and Body Weight Gain[a]

Observation	Week	Dose Level (ppm)			
		0	0.5	5.0	150
MALES					
Body Weight (g)	0	266.4	268.1	266.6	266.4
	1	295.4	300.9	296.9	277.4** (-6.5)
	13	421.8	434.4	435.6	442.2 (+4.8)
Gain (g)					
	0-1	28.9	32.8**	30.4	11.0**
	0-2	49.1	57.1**	52.6	40.9**
	0-13	155.4	166.3	169.1	175.8
FEMALES					
Body Weight (g)	0	177.1	176.9	178.1	175.6
	1	191.3	193.1	192.5	179.9** (-6.0)
	13	263.8	261.9	262.9	262.5 (-0.5)
Gain (g)	0-1	14.2	16.2	14.3	4.2**
	0-13	86.6	84.4	84.8	86.9

[a] Data summarized from Table 3, 4, 5, and 6 (pp 21, 23, 24, and 26) of the study.
** $p \leq 0.01$

D. Food Consumption and Achieved Dosage

1. Food Consumption: Mean food consumption data are summarized in Table 4. Mean food consumption of high-dose animals was significantly lower than controls for Week 0-1 in males and females. Food consumption for high-dose animals returned to control levels at Week 1-2 and all other intervals, thereafter, through the end of the study.

Table 4: Mean Food Consumption (grams/animal/day)[a]

Sex	Week	Dose Level (ppm)			
		0	0.5	5.0	150
Males	0-1	22.4	22.7	22.6	17.2**
	1-2	22.1	22.5	22.5	22.8
Females	0-1	16.5	16.8	16.3	12.5**
	1-2	16.7	16.4	16.3	17.1

[a] Data summarized from Table 3, 4, 5, and 6 (pp 21, 23, 24, and 26) of the study.
** $p \leq 0.01$

prepared from adequately perfused and properly dissected nervous system tissues, artifactual changes such as vacuolation or myelin sheath swelling may be present. Also, some variations exists from animal to animal and from section to section in the amount of endoneurial connective tissue present within the peripheral nerves."

3. **Neuropathology Positive Controls:** Not included with study.

Table 6: Summary of FOB Findings[a]

Observation		Dose Level (ppm)			
		0	0.5	5.0	150
MALES					
Urine, None	Prestudy	7/10	7/10	7/10	5/10
	Week 4	5/10	4/10	1/10	0/10*
	Week 9	5/10	6/10	3/10	1/10
	Week 13	5/10	5/10	6/10	1/10
Startle Response, Exaggerated	Prestudy	1/10	0/10	1/10	1/10
	Week 4	0/10	0/10	0/10	4/10
	Week 9	0/10	0/10	1/10	2/10
	Week 13	0/10	0/10	0/10	2/10
Tail Pinch Response, Exaggerated	Prestudy	3/10	1/10	2/10	1/10
	Week 4	0/10	0/10	0/10	4/10
	Week 9	0/10	0/10	1/10	2/10
	Week 13	0/10	0/10	0/10	0/10
Body Weight (g)	Prestudy	229.9	228.1	235.4**	229.1
	Week 4	331.8	350.0**	352.2**	337.0
	Week 9	394.4	411.5*	418.7**	410.9*
	Week 13	423.2	437.7	450.5**	447.2*
FEMALES					
Startle Response, Exaggerated	Prestudy	0/10	0/10	0/10	0/10
	Week 13	0/10	0/10	0/10	1/10
Forelimb Grip Strength (kg)	Prestudy	0.52	0.47	0.50	0.57
	Week 13	0.61	0.68	0.70	0.88*

[a] Data summarized from study Tables 11, 12, 13, 14, 15, and 18 (pp 31-45).
* $p \leq 0.05$, ** $p \leq 0.01$

FOB findings revealed minimal effects in high-dose animals at the Weeks 4, 9 and 13 evaluations. High-dose males had a decreased incidence of no urination and an increased incidence of exagerated tail pinch responce. High-dose males and females had an increased incidence startle responses in the manipulative observations. High-dose females had increased forelimb grip strength at Week 13. The mean body weights of treated males were significantly greater than the concurrent control values.

Necropsy findings did not reveal any treatment-related gross pathological or histopathological findings. Although histopathological lesions were observed, incidences were low and attributed by the study pathologist to animal variation and artifactual changes.

VI. REVIEWER'S COMMENTS and CONCLUSIONS

The dosages selected in this subchronic neurotoxicity study appeared to be adequate to evaluate the neurotoxic effects of Fipronil. Although the neurobehavioral effects in this study appeared to be borderline at the high-dose tested (150 ppm), they are consistent with clinical signs observed in a chronic toxicity study/oncogenicity study in rats (MRID No.: 429186-48, HED Doc. No.: 011086) submitted by the Registrant.

The FOB evaluations from the subchronic neurotoxicity study indicated that the rats were hyperexcitable with exaggerated startle and tail pinch responses observed in males and/or females. For the first 13 weeks of the chronic toxicity study, hyperexcitable behavior was also observed and summarized in Table 8. Clinical signs of rats dosed at 300 ppm (12.68 mg/kg/day, males; 16.75 mg/kg/day, females) included the following behavioral changes: aggressiveness, irritability, and hyperactivity and excessive vocalizations (females). Convulsions were observed in males, and to a lesser extent in females. Dosing the rats at 300 ppm also produced borderline toxicity, with slight decreases in mean body weight (92%, males; 95% females) and body weight gain (89%, males; 93%, females), and increased incidence in mortality (4/80, males; 1/80, females).

Although the neurobehavioral effects observed in the neurotoxicity study are slight, taken in conjunction with the results at 300 ppm of the chronic toxicity study, the high-dose tested (150 ppm) appears to be a valid LOEL for neurobehavioral effects.

Based on the results (FOB findings) of this study, the LOEL was established at 150 ppm (8.89 mg/kg/day, males; 10.8 mg/kg/day, females); the NOEL was established at 5.0 ppm (0.301 mg/kg/day, males; 0.351 mg/kg/day, females).

This study is classified as Core - Acceptable and satisfies guideline requirements (§82-7) for a subchronic neurotoxicity screening battery in the rat.