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WASHINGTON, DC 20460



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HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

December 11, 2001

TXR No. 0050331

MEMORANDUM

SUBJECT: Acetamiprid - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan *Sanjivani Diwan*
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Pamela Hurley, Toxicologist
Michael Doherty, Risk Assessor
Registration Action Branch 2
Health Effects Division (7509C)

And

Akiva Abramovitch, Product Manager
Insecticide and Rodenticide Branch
Registration Division (7505C)

The Cancer Assessment Review Committee met on October 31, 2001 to evaluate the carcinogenic potential of Acetamiprid. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

TXR NO. 005033I

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF
ACETAMIPRID
PC. Code: 099050

FINAL REPORT

December 11, 2001

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION:

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DATA PRESENTATION:

Pamela Hurley
Pamela Hurley, Toxicologist

DOCUMENT PREPARATION:

Sanjivani Diwan
Sanjivani Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

William Burnam

W. Burnam

Marion Copley

Marion Copley

Virginia Dobozy

Virginia Dobozy

Yiannakis Ioannou

Y. M. Ioannou

Tim McMahon

T. McMahon

Nancy McCarroll

Nancy McCarroll

Esther Rinde

(Minority Report Attached)

Joycelyn Stewart

Joycelyn Stewart

Clark Swentzel

C. Swentzel

Yin-Tak-Woo

See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher Pathology Consultant

See attached sheet

Lori Brunzman, Statistical Analysis

Lori Brunzman

The data package was prepared by Artensie Flowers/ OPP/HED. The meeting was attended by Artensie Flowers/OPP/HED, Linnea Hansen/OPP/HED and by conference call, Gordon Cockell/Pesticide Management Regulatory Agency (PMRA), Canada and toxicologists at California/Environmental Protection Agency (CAL/EPA).

Acetamiprid Cancer Assessment Document Final Report

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Lori Brunsman, Statistical Analysis

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Acetamiprid

Cancer Assessment Document

Final Report

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DOCUMENT PREPARATION:

Sanjivani Diwan, Executive Secretary

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Lori Brunsman, Statistical Analysis

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EXECUTIVE SUMMARY

On October 31, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Program met to evaluate the carcinogenic potential of acetamiprid. This meeting was held jointly with Pesticide Management Regulatory Agency (PMRA), Health Canada, Canada and California/Environmental Protection Agency (CAL/EPA).

Acetamiprid was administered in the diet to male and female Crl: CD (BR) rats at 0, 160, 400 and 1000 ppm (0, 7.1, 17.5 and 46.4 mg/kg/day for males and 0, 8.8, 22.6, and 60.0 mg/kg/day for females) for 24 months; and to male and female Crl: CD -1 (ICR) BR mice at 0, 130, 400 and 1200 ppm (0, 20.3, 65.6 and 186.3 mg/kg/day for males and 0, 25.5, 75.9 and 214.6 mg/kg/day for females, respectively) for 78 weeks.

The CARC concluded that acetamiprid was not carcinogenic to male and female rats and mice based on the following:

- Male rats had a significant increasing trend ($p < 0.05$) in interstitial cell tumors of the testes but there were no significant differences in the pair-wise comparisons of the dosed groups with the controls. The incidence at the high dose (1000 ppm) was within the historical control range for Charles River Laboratories. **The CARC considered that testicular tumors are common to this strain of rat and concluded that the increase in testicular tumors was not treatment-related.**
- For female rats, there were statistically significant differences ($p < 0.05$) in the pair wise comparisons of the 400 ppm dose group with the controls for pituitary adenomas and combined adenomas/adenocarcinomas. However, no increase in these tumors was noted at the high dose (1000 ppm). These incidences did not exceed the available historical control range for this tumor. **Therefore, the increased incidences at 400 ppm were not considered by the CARC to be treatment-related.** Female rats had a significant increasing trend in adenocarcinomas (borderline; $p = 0.054$) and combined mammary gland adenomas/adenocarcinomas ($p < 0.05$). The increase in the incidence of combined mammary gland adenomas/adenocarcinomas was driven by adenocarcinomas. The incidences of mammary gland adenomas in the mid- and high dose groups (400 and 1000 ppm, respectively) were within the historical control range for Charles River Laboratories. Although the incidence of adenocarcinomas for mid- and high-dose groups exceeded the historical control range of the testing laboratory, it was within the range of values from the supplier and did not reach statistical significance by pair wise comparison with the controls. The incidence of adenocarcinomas did not exceed the range observed in Charles River historical control data. The increased incidence of mammary tissue hyperplasia was seen only in high-dose females with no increase in severity and there was no increase in the incidence of galactocoele in the mammary tissue. **The Committee,**

therefore, concluded that the increases in these tumors were not treatment-related. Dosing at the highest level was considered to be adequate and not excessive based on a decrease in body weight gain in males (18%) and females (23%), decrease in food consumption in both sexes as well as renal changes and liver hypertrophy and vacuolation in males.

- There was no treatment-related increase in any tumors in male and female mice. Dosing at the highest dose (1200 ppm) was considered by the CARC to be adequate and not excessive based on decrease in body weight gain in males and females (12% and 11%, respectively at 13 weeks and 55% and 58%, respectively, at 78 weeks).
- In an *in vitro* assay, acetamiprid was clastogenic to Chinese Hamster Ovary cells. However, both acetamiprid and its metabolites, IM-1-4, IM-1-2, IM-0 and IC-0, were not mutagenic in *in vitro* and/or *in vivo* assays. The available studies satisfy pre-1991 FIFRA guideline requirements. At this time, the Committee has no concern for mutagenicity of acetamiprid.
- A structurally-related compound, imidacloprid, is not mutagenic and has been classified as category "E" (not carcinogenic to humans). A second more distantly related compound, thiamethoxam, is not mutagenic but has been classified as "Likely to be carcinogenic to humans based on an increased incidence of liver tumors in mice."

P The majority of the members concluded that acetamiprid was not likely to be carcinogenic to humans because: there was 1) an absence of a dose-response and a lack of a statistically significant increase in the mammary adenocarcinoma incidence by pair wise comparison of the mid- and high-dose groups with the controls; although the incidence exceeded the historical control data from the same lab, it was within the range of values from the supplier; also the control incidences in this study were on the high side; 2) a lack of an increase in the incidence of galactocoele in the mammary tissue; and 3) an increased incidence of mammary tissue hyperplasia noted in high-dose females but not in mid-dose females.

Three members believed that the mammary tumor response, although marginal, cannot be discounted because: 1) there was an increase in the incidence of adenocarcinomas at the two top doses and the incidence exceeded the historical control range based on three studies from the testing laboratory and 2) the combined incidence of mammary adenomas/adenocarcinomas was driven by adenocarcinoma, a malignant tumor (see attachment for rationale supporting classification as "Suggestive..."). One member felt that the data were inadequate to assess the carcinogenic potential of acetamiprid because: 1) the appropriate historical control data were not available for relevant comparison, and 2) the animals could have tolerated a higher dose.

Based on the majority opinion, and in accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified acetamiprid into the category "Not likely to be carcinogenic to humans" and, therefore, the quantification of human cancer risk is not required.

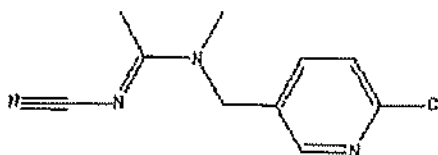
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I. INTRODUCTION

On October 31, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Program met to evaluate the carcinogenic potential of acetamiprid. Acetamiprid has not been previously reviewed by the CARC for its carcinogenicity. This review was conducted jointly with PMRA, Canada, along with participation of CAL/EPA. Dr Pamela Hurley, Acting Branch Chief, Registration Branch 2/HED, presented the chronic/carcinogenicity studies in Wistar Crl:CD® BR rats and B6C3F1 mice by: describing the experimental design; reporting on survival and body weight effects, treatment-related non-neoplastic and neoplastic lesions, statistical analysis of the tumor data, the adequacy of the dose levels tested; and presenting the weight of the evidence for the carcinogenicity of acetamiprid. Dr. Hurley also discussed the toxicology, metabolism and mutagenicity studies as well as structure-activity relationships of the related compounds.

II. BACKGROUND INFORMATION

Acetamiprid is a new active ingredient in the neonicotinoid class of insecticides which acts as an agonist of the nicotinic acetylcholine receptor (nACh) of the postsynaptic membrane of nerve cells. The active ingredient interrupts the function of the insect nervous system. Biochemical radio ligand binding studies show that acetamiprid interacts with high affinity at the insect nAChR binding site and with low affinity at the vertebrate nAChR. The differences in the affinities of acetamiprid at the insect and vertebrate nAChR may indicate that there are structural differences between insect and vertebrate nAChRs, and may account for acetamiprid's selective toxicity to insects. Acetamiprid is structurally related to imidacloprid. Acetamiprid's PC Code is 099050 and its CAS number is 135410-20-7. The structure of acetamiprid is given below.



Acetamiprid

Acetamiprid is proposed to be used on a wide range of crops and plants, including seed treatments (canola and mustard seed), cotton, leafy and fruiting vegetables, citrus and pome fruits, cole crops, grapes, plants and flowers and ornamentals. Some of these uses will be residential uses.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with NI-25 (acetamiprid) in Crl:CD® BR Rats

Reference: R.C. Hatch (1999) Two year dietary toxicity and oncogenicity study in rats. MPI

Research, Inc., Mattawan, MI, Study No. 449-015, September 28, 1999. MRID 44988429 & 45245304. Unpublished.

A. Experimental Design

NI-25 (>99% a.i.; Lot No. NNI-01) was administered to groups of 60 male and 60 female Crl:CD® BR rats in the diet at concentrations of 0, 160, 400, and 1000 ppm (0, 7.1, 17.5, and 46.4 mg/kg/day for males and 0, 8.8, 22.6, and 60.0 mg/kg/day for females). Ten rats per sex per dose were sacrificed at 12 months for interim evaluations; the remaining animals were maintained on their respective diets for up to 24 months.

B. Discussion of Tumor Data

Survival Analysis

The statistical evaluation of mortality indicated no statistically significant incremental changes with increasing doses of acetamiprid in male or female rats.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analysis

The statistical analyses of the male and female rats were based upon the Exact trend test and the Fisher's Exact test for pair wise comparisons. See Tables 1 through 3 for the tumor analyses results.

Male rats had a significant increasing trend in testes interstitial cell tumors at $p < 0.05$. There were no significant differences in the pair wise comparisons of the dosed groups with the controls. The testes interstitial cell tumor incidence was 1/47, 2/50, 0/48, and 5/48 (2%, 4%, 0% and 10%) for 0, 160, 400, and 1000 ppm, respectively. The incidence at the highest dose was within the historical control range (1.4%-10%) for Charles River laboratories (992). Historical control data from the testing laboratory were not provided.

Female rats had a significant increasing trend in combined mammary gland adenomas/adenocarcinomas ($p < 0.05$). Although the trend for adenocarcinomas was of borderline significance ($p=0.05$), there were no significant pair wise comparisons for either adenomas, adenocarcinomas or combined adenomas/adenocarcinomas. There was no significant increasing trend for adenomas. The incidences of mammary adenomas for the mid-dose and high-dose females (7% and 5%, respectively) were within the historical control range (2%-32%) for Charles River Laboratories (1992). The incidences of mammary adenocarcinomas were 10/59, 11/60, 16/59, and 17/60 (17%, 18%, 27% and 28%, respectively). The incidence of 27% for the mid-dose and 28% for the high-dose groups exceeded the historical control, but did not reach

statistical significance compared to concurrent controls. Historical control data were provided for mammary adenocarcinomas for three dietary studies (range of 14%-18%) and 4 gavage studies (range of 13-29%). This study was a dietary study. The combined incidence of adenomas/adenocarcinomas (32%); driven by adenocarcinomas; was within the historical control range (8.6%-58.3%) for Charles River laboratories (1992).

In female rats, there were significant differences in the pair wise comparisons of the 400 ppm dose group with the controls for pituitary adenomas and combined adenomas/adenocarcinomas, both at $p < 0.05$ but not at the high dose of 1000 ppm. The incidences of pituitary tumors at 400 and 1000 ppm were as follows: adenomas : 48/59 (81%) and 44/60 (73%) vs 38/60 (63%) in controls; and combined adenomas/adenocarcinomas : 50/59 (85%) and 46/60 (77%) vs 39/60 (65%) in controls. The combined incidence was driven by adenomas. The incidence of pituitary adenomas at 400 ppm was within the historical controls range (31.4%-88.8%) for Charles River Laboratories. Historical control data from the testing laboratory were not provided.

Table 1. Male Rats: Testes Interstitial Cell Tumor Rates and Exact Trend Test and Fisher's Exact Test Results (Brunsmann, 2001).

ppm	0	160	400	1000
mg/kg/day	0	7.1	17.5	46.4
Tumor Type				
Interstitial ^a	1/47	2/50	0/48	5 ^b /48
(%)	(2)	(4)	(0)	(10)
p =	0.0261 [*]	0.5234	0.4947	0.1067

Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 54.

^{*} $p < 0.05$

^a Historical control data for benign interstitial cell tumors of the testes in Charles River Sprague Dawley rats range from 1.4-10% with a mean of 4.68% in Charles River Laboratories (published 1992).

^b First tumor observed at week 85, dose 1000 ppm

Table 2. Female Rats: Pituitary Tumor Rates and Exact Trend Test and Fisher's Exact Test Results (Brunsman, 2001).

ppm	0	160	400	1000
mg/kg/day	0	8.8	22.6	60.0
Tumor Type				
Adenomas ^a (%) p =	38 ^b /60 (63) 0.1377	41/59 (69) 0.3028	48/59 (81) 0.0227*	44/60 (73) 0.1633
Adenocarcinomas (%) p =	1/60 (2) 0.1720	0/59 (0) 0.5042	2 ^c /59 (3) 0.4936	2/60 (3) 0.5000
Combined (%) p =	39/60 (65) 0.0731	41/59 (69) 0.3722	50/59 (85) 0.0111*	46/60 (77) 0.1140

Number of tumor bearing animals/number of animals examined, excluding those that died before week 43.

^a Historical control data for pituitary adenomas in female Charles River Sprague Dawley rats range from 31.4-88.8% with a mean of 72.1% in Charles River Laboratories (published 1992).; * p < 0.05

^b First adenoma observed at week 53, dose 0 ppm.

^c First adenocarcinoma observed at week 43, dose 400 ppm.

Table 3 Female Rats: Mammary Gland Tumor Rates and Exact Trend Test and Fisher's Exact Test Results (Brunsman, 2001).

ppm	0	160	400	1000	Historical Control	
mg/kg/day	0	8.8	22.6	60.0		
Tumor Type					Range (%)	Mean (%)
Adenomas (%) p =	1/59 (2) 0.0888	0/60 (0) 0.4958	4/59 (7) 0.1821	3 ^a /60 (5) 0.3157	2-32 ^f	8
Adenocarcinomas (%) p =	10/59 (17) 0.0543	11 ^b /60 (18) 0.5171	16/59 (27) 0.1333	17/60 (28) 0.1029	14-18 ^c 9-58 ^f	16 24
Combined (%) p =	11/59 (19) 0.0294 [*]	11/60 (18) 0.5760	17 ^e /59 (29) 0.1396	19 ^d /60 (32) 0.0768	8.6-58.3% ^f	

Number of tumor bearing animals/number of animals examined, excluding those that died or were sacrificed before week 31

^a First adenoma observed at week 53, dose 1000ppm

^b First adenocarcinoma observed at week 31, dose 160ppm

^c Three animals in the 400 ppm dose group had both an adenoma and an adenocarcinoma

^d One animal in the 1000 ppm dose group had both an adenoma and adenocarcinoma

^e Historical control data from same testing laboratory (three studies performed from 7/7/1992-7/6/1994, 4/22/1993-4/27/1995, and 7/28/1994-7/31/1996).

^f Historical control data from Charles River Laboratories (published in 1992)

* p < 0.05

C. Non-Neoplastic Lesions (Refer to Table 4)

Mammary Lesions

In the 12-month sacrifice group, the incidence of mammary galactocele (milk containing cyst) (54%) was significantly increased in mid-dose females, but not in control, low-dose or high-dose females. In the main study, the incidence of mammary hyperplasia (not otherwise specified; (49%, p<0.05)) was increased in high-dose females compared with the high control incidence of 29%. There was no notable increase in the severity of mammary hyperplasia except that the lesion was moderate in one female each from the mid- and high-dose groups compared with none in the controls. The incidence of mammary hyperplasia in historical controls in female rat studies at the testing laboratory ranged from 5-59% across seven studies. The increased incidence of mammary galactocele in mid-dose females sacrificed at 12 months is not considered treatment related because no statistical increase was observed for high-dose females, and the incidence was very high at all dose levels in the main study group. The incidence of mammary hyperplasia was significantly increased in high-dose females in the main study. The study author did not further describe this lesion. The study author also noted that the incidence was not significantly increased when the interim sacrifice and main study animals were combined for statistical analysis. The incidence of 49% of mammary hyperplasia in high-dose females was less than the

historical control upper range of 59%, which appears to be an outlier, because it was almost three times greater than the next highest incidence of 20%. The study author concluded that the toxicologic significance of mammary hyperplasia was uncertain. The reviewer concludes that mammary hyperplasia may be related to the mammary neoplasms; however hyperplasia did not show a dose-related trend similar to that of the mammary adenocarcinomas.

Liver and Other Lesions:

In the 12-month sacrifice group, hepatocellular hypertrophy was observed in 42% ($p < 0.05$) of mid-dose and 91% ($p < 0.01$) of high-dose male rats. Hepatocellular hypertrophy was observed in 36% ($p < 0.05$) of high-dose female rats. Hepatocellular vacuolation was observed in 83% ($p < 0.01$) of mid-dose and 91% ($p < 0.01$) of high-dose group male rats compared with 17% of control and 40% (N.S.) of low-dose group male rats.

In the main study (those surviving longer than 12 months), the incidences of hepatocyte hypertrophy and hepatocyte vacuolation were significantly ($p < 0.01$) increased in the mid- and high-dose male rats groups. The incidences in mid- and high-dose males were 31% and 71%, respectively, for hypertrophy and 46% and 60%, respectively, for vacuolation. In addition, micro concretions in the renal papilla were observed in 76% ($p < 0.01$) of high-dose males compared with 35% of controls.

Alveolar macrophages were found in the lungs of 31% ($p < 0.05$) of high-dose females compared with only 12% of the controls.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on significantly decreased mean body weight gain when compared to the control groups in both sexes at 1000 ppm and an increased incidence of hepatocyte hypertrophy and vacuolation as well as microconcretion in renal papilla in male rats at the highest dose tested (1000 ppm, 46.4/60.0 mg/kg/day (M/F)). High-dose male rats weighed 10-13% ($p < 0.01$) less than controls throughout the study and gained 18% less over the entire

study when compared to the control group. High-dose group males also consumed 4-9% ($p < 0.01$ or < 0.05) less food at different time points throughout the study. High-dose females weighed 6-27% ($p < 0.01$) less than the control group during the study and gained 23% less over the entire study. Food consumption was 9-19% less for most of the study. Microscopic lesions of the liver and kidney were also observed in mid- and high-dose animals (see page #6).

TABLE 4 Notable microscopic findings in male and female rats fed NI-25 for up to 24 months				
Organ/Lesion	Dietary Concentration (ppm)			
	0	160	400	1000
Males – 12 months				
Liver [No. animals examined]	[12]	[10]	[12]	[11]
Hypertrophy	0	0	5* (1.00) ^a	10** (1.20)
Hepatocellular vacuolation	2 (1.00)	4 (1.50)	10** (1.10)	10** (1.20)
Males – Main study				
Kidney [No. animals examined]	[48]	[50]	[48]	[49]
Microconcretion, papilla	17 (1.06)	23 (1.09)	23 (1.35)	37** (1.41)
Liver [No. animals examined]	[48]	[50]	[48]	[48]
Hypertrophy	0	0	15** (1.00)	34** (1.35)
Hepatocyte vacuolation	10 (1.5)	9 (1.33)	22** (1.23)	29** (1.41)
Females – 12 months				
Liver [No. animals examined]	[10]	[11]	[13]	[11]
Hypertrophy, trace	0	0	0	4*
Hepatocyte vacuolation	1	0	0	1
Mammary [No. animals examined]	[10]	[11]	[13]	[11]
Galactocele	1 (1.00)	1 (1.00)	7** (2.57)	4 (1.75)
Females – Main study				
Liver [No. animals examined]	[50]	[49]	[47]	[49]
Hypertrophy, trace	0	0	0	3
Hepatocyte vacuolation	9 (1.67)	9 (1.67)	8 (1.50)	5 (1.00)
Lung [No. animals examined]	[50]	[49]	[47]	[49]
Alveolar macrophage	6 (1.33)	6 (1.00)	8 (1.13)	15* (1.40)
Mammary [No. animals examined]	[49]	[49]	[47]	[49]
Galactocele	28 (1.96)	29 (2.03)	33 (2.00)	29 (2.07)
Hyperplasia	14 (1.21)	12 (1.17)	14 (1.36)	24* (1.38)

^a Average severity grade: 1 = trace, 2 = mild, 3 = moderate, and 4 = severe, calculated by the reviewer.

*p<0.05, **p<0.01, statistically significant, treated groups compared with controls.

In the 13-week feeding study in rats, 800-ppm males and females gained 13% and 21% less weight than controls, respectively (n.s.), resulting in final body weights 91% and 89% of controls, respectively (n.s.). Decreased food consumption levels (g/animal/day) were observed at various times during the study (80-91% of the controls); however, no statistically significant differences were observed in mean food efficiencies. At 1600 ppm (HDT: 99.9/117.1 mg/kg/day (M/F), males and females had decreases in terminal mean absolute body weights at 87% (p<0.05) and 79% (p<0.01) of controls, respectively. Mean body weight gains for the treatment period of weeks 1-13 were 80% (p<0.05) and 59% (p<0.01) of controls, respectively. Decreased food

consumption levels (g/animal/day) were observed in both sexes throughout the study (73-91% of controls) and decreased mean food efficiencies were observed at several times during the study. Increased levels of total cholesterol were observed in males (141% of controls; $p < 0.01$) and females (124% of controls, n.s.). Microscopic examination of the liver revealed centrilobular hypertrophy in 10/10 males fed 800 or 1600 ppm and 8/10 and 10/10 females fed 800 or 1600 ppm, respectively, with the mean severity of the lesion graded as 1.8 and 3.0, respectively, for males and 1.0 and 1.9, respectively, for females. This lesion was not observed in any of the other treated animals or in the controls.

Based on the results from the chronic/carcinogenicity study in rats and the subchronic feeding study in rats, the high dose level of 1000 ppm in the chronic/carcinogenicity study was considered by the CARC to be adequate.

♀ 2. Carcinogenicity Study in CrI:CD-1@ (ICR) BR Mice

Reference: Goldenthal, E.I. (1999) 18-Month dietary oncogenicity study in mice. MPI Research, Inc., 54943 North Main Street, Mattawan, MI 49071-9399. Laboratory project ID.: 449-016, September 17, 1999, MRID 44988428. Unpublished.

Cunny, H.C. (2000) Supplemental historical background data for the acetamiprid 18-month study in mice - MRID 44988428. Aventis CropScience, P.O. Box 12014, Research Triangle Park, NC 27709 and Nippon Soda Co., Ltd., Agro Products Division, 2-1 Ohtemachi 2-Chome, Chiyoda-ku, Tokyo 100-8165, Japan. October 16, 2000, MRID 45245305. Unpublished.

A. Experimental Design

Acetamiprid (99.7% a.i., Lot #: NNI-01) was administered to groups of 50 male and 50 female CrI:CD-1@ (ICR) BR mice in the diet at concentrations of 0, 130, 400, or 1200 ppm for up to 78 weeks. An additional, 10 males and 10 females at each dietary concentration were terminated after 52 weeks for interim evaluation. Time-weighted average doses were 20.3, 65.6, and 186.3 mg/kg/day, respectively, for males and 25.2, 75.9, and 214.6 mg/kg/day, respectively, for females.

B. Discussion of Tumor Data

A summary of common neoplasms seen at terminal sacrifice in this study is given in Table 5. No significant treatment-related increases in neoplasms were found in the study. The most commonly found neoplasms were in the liver and lungs of males and in the lungs of females with the incidence rates for all tumors within the range of the historical data. It should be noted that the historical data included studies conducted between January 1987 and December 1996 and, therefore, included studies conducted after the current study was completed.

C. Non-Neoplastic Lesions

For animals that were sacrificed at study termination, centrilobular hepatocellular hypertrophy was observed in 23/39 high-dose males ($p \leq 0.01$; severity = 1.17), in 3/38 mid-dose females (n.s.; severity = 1.00), and in 16/43 high-dose females ($p \leq 0.01$; severity = 1.19), but in none of the animals from the control or other dose groups. The incidence rates of myeloid hyperplasia of the bone marrow in the femur and sternum were significantly ($p \leq 0.05$ or 0.01) increased for all treated males and for low- and high-dose females as compared with the controls (Table 6).

In males at terminal sacrifice, the incidence rate of amyloidosis was significantly ($p \leq 0.05$ or 0.01) increased for the high-dose group in numerous organs (adrenal cortex, jejunum, kidney, liver, nonglandular stomach, testis, and thyroid gland) as shown in Table 6. In addition, the incidence rate of amyloidosis was significantly ($p \leq 0.05$) increased for the adrenal cortex and kidney of the mid-dose males.

Among females in the control, low-, mid-, and high-dose groups, chronic progressive nephropathy was observed in 21/38, 24/42, 21/38, and 35/43 ($p \leq 0.05$) animals, respectively (severity = 1.00-1.08 for all groups), and epithelial hyperplasia in the lung was observed in 0/38, 4/42, 1/38, and 5/43 ($p \leq 0.05$) animals, respectively.

TABLE 5. Neoplastic findings in male and female mice fed acetaminiprid for up to 78 weeks and historical incidences^a				
Organ or tissue / neoplasm	0 ppm	130 ppm	400 ppm	1200 ppm
Males				
No. Examined 12 mo. - termination	48	49	47	43
No. Examined 0 -12 mo.	12	11	13	17
Liver / hepatocellular adenoma	7 (14.58%) ^b	2 (4.08%)	2 (4.26%)	0
Historical incidence: 10.46% (2.86-28.00%)				
Liver / hepatocellular carcinoma	1 (2.08%)	0	0	1 (2.33%)
Historical incidence: 5.29% (1.54-16.00%)				
Lung / bronchiolar adenoma ^c	13 (21.67%)	11 (18.33%)	5 (8.33%)	4 (6.67%)
Historical incidence: 14.29% (2.00-42.00%)				
Lung / bronchiolar carcinoma	0	1 (2.04%)	0	1 (2.33%)
Historical incidence: 6.87% (1.43-26.00%)				
Females				
No. Examined 12 mo. - termination	47	49	44	47
No. Examined 0 -12 mo.	13	11	16	13
Liver / hepatocellular adenoma	0	1 (2.04%)	0	0
Historical incidence: 0.99% (0.85-7.84%)				
Liver / hepatocellular carcinoma	0	0	0	0
Historical incidence: 0.66% (1.43-4.29%)				
Lung / bronchiolar adenoma ^c	9 (15.00%)	10 (16.67%)	11 (18.33%)	4 (6.67%)
Historical incidence: 8.51% (1.67-26.67%)				
Lung / bronchiolar carcinoma	1 (2.13%)	0	0	0
Historical incidence: 4.08% (0.77-18.37%)				

a Historical control data submitted by MPI Research, 2001 (from January 1987 and December 1996)

b Percent incidence

TABLE 6 Incidences of selected non-neoplastic microscopic findings in male and female mice fed acetaminiprid for up to 78 weeks				
Organ/finding	0 ppm	130 ppm	400 ppm	1200 ppm
Males				
Number examined (terminal sacrifice)	37	42	37	39
Liver - hypertrophy	0	0	0	23**
Femur - myeloid hyperplasia	0	5*	7**	6*
Sternum - myeloid hyperplasia	0	6*	7**	6*
Adrenal cortex - amyloidosis	0	3	5*	7**
Jejunum - amyloidosis	1	n/e	n/e	7*
Kidney - amyloidosis	0	3	5*	7**
Liver - amyloidosis	0	3	3	5*
Nonglandular stomach - amyloidosis	0	n/e	n/c	5*
Testis - amyloidosis	0	2	2	5*
Thyroid gland - amyloidosis	0	3	3	5*
Females				
Number examined	38	42	38	43
Liver - hypertrophy	0	0	3	16**
Femur - myeloid hyperplasia	0	5*	4	6*
Sternum - myeloid hyperplasia	0	6*	4	6*

Significantly different from control: * $p \leq 0.05$; ** $p \leq 0.01$.

n/e = not examined

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on decreased body weight gain and microscopic lesions of the kidney, liver and bone marrow in the high-dose group. High-dose males and females had significantly ($p \leq 0.01$) lower absolute body weights, which ranged from 83-93% and 82-91% of the control levels, respectively throughout the study. Food consumption (g/animal/day) by the high-dose males and females was significantly ($p \leq 0.05$ or 0.01) less than that of the controls at most intervals throughout the study. In males surviving to terminal sacrifice, the incidence rate of amyloidosis was significantly ($p \leq 0.05$ or 0.01) increased in numerous organs (see Table 6).

In the subchronic feeding study in the mouse, two treatment-related deaths/sex were observed at the highest dose level of 3200 ppm (430.4/466.3 mg/kg/day (M/F)). Tremors were observed in 5/10 females at this dose level during weeks 4-13. Weekly absolute body weights for the 3200-ppm males and females ranged from 65-79% and 64-77%, respectively, of the control group levels and attained statistical significance ($p \leq 0.01$) beginning at week 1. Overall weight change by the 3200-ppm males and females resulted in a net weight loss by both sexes and was significantly ($p \leq 0.001$) less than that of the controls. Males in the 3200 ppm group had

significantly ($p \leq 0.01$; 64-75% of controls) reduced weekly food consumption values throughout the study as compared with the controls except for weeks 3 and 12. Food consumption by the 3200-ppm females was also significantly ($p \leq 0.01$; 65-73% of controls) less than that of the controls throughout the study. Weekly food efficiencies for the 3200-ppm groups were often negative values and generally less than those of the controls with statistical significance ($p \leq 0.05$ or 0.01) attained at some weeks. Absolute body weights for the 1600-ppm (211.1/249.1 mg/kg/day (M/F)) males and females were significantly ($p \leq 0.05$; 82-91% of controls) less than the controls beginning at weeks 3 and 1, respectively. Overall body weight gains by the 1600-ppm males and females were 19% and 21%, respectively, of the control levels ($p \leq 0.05$). In the 1600- and 3200-ppm males and females differences in clinical chemistry parameters, histopathological lesions, and organ weights were indicative of inanition.

Based on the results from the carcinogenicity study in mice and the subchronic feeding study in mice, the high dose level of 1200 ppm in the carcinogenicity study was considered to be adequate by the CARC.

IV. TOXICOLOGY

1. Metabolism

NI-25 (acetaminiprid) is extensively and rapidly metabolized by rats. Metabolites accounted for 79-86% of the administered radioactivity and profiles were remarkably similar for males and females and for both oral and intravenous dosing. Only 3-7% of the dose was recovered in the urine and feces as unchanged test article. Urinary and fecal metabolites from the 15-day repeat dose experiment were also characterized and showed minor differences from the single-dose test groups, the most relevant of which was a slight increase (10.1% of dose for males and 10.3% of dose for females vs <4% in the single dose groups) in the glycine conjugate (IC-O-gly). The initial Phase I biotransformation appears to be demethylation of the parent compound resulting in a major metabolite, IM-2-1. The most prevalent metabolite, IC-O (6-chloronicotinic acid) results from the removal of the cyanoacetamide group from the demethylated IM-2-1. In the repeat-dose study, it appeared that the results of Phase II metabolism became more easily detectable as shown by the increase in the glycine conjugate, IC-O-gly. A metabolism pathway was proposed by the study authors of the ADME study that is consistent with available data from the reviewed studies (MRIDs: 44988503-44988507).

2. Mutagenicity

Acetaminiprid- (parent compound)

In a reverse gene mutation assay, *in vitro* mammalian cell gene mutation test, *in vivo* chromosomal aberration assay, mouse micronucleus assay, and repeat unscheduled DNA synthesis assay, acetaminiprid was found to be nonmutagenic. However, in an *in vitro* chromosomal aberration assay, acetaminiprid was found to be clastogenic to Chinese hamster ovary

cells with or without metabolic activation. No mutagenicity studies were found in the open literature for acetamiprid.

- (1) In a repeat reverse gene mutation assay, when tested in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and the WP2 uvrA (tryptophane auxotroph, try) strain of *Escherichia coli* at concentrations up to 5000 µg/plate, NI-25 (Acetamiprid) was non mutagenic with or without metabolic activation. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data (MRID No. 44651849).
- (2) In a mammalian forward cell gene mutation assay with Chinese hamster ovary cells, acetamiprid at concentrations ranging from 500 µg/ml to 4000 µg/ml was nonmutagenic with or without metabolic activation. This study is classified as acceptable and satisfies the FIFRA Test Guideline for mammalian cell gene mutation data (MRID No. 44651857).
- (3) In an *in vitro* mammalian chromosome aberration assay, acetamiprid was found to be clastogenic to Chinese hamster ovary cells *in vitro* with or without metabolic activation. Effects with metabolic activation were significant and dose-related. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline for *in vitro* cytogenetic data (MRID No. 44651855).
- (4) In an *in vivo* chromosome aberration assay, there was no significant dose-related increase in chromosome aberrations in bone marrow following a 250 mg/kg oral dose of acetamiprid. Although only one NI-25 dose was assayed, there was clear evidence of toxicity (death and other clinical signs) to the treated animals and cytotoxicity (i.e. reduced mitotic activity). This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data (MRID No. 44651854).
- (5) In a mouse micronucleus assay no increase in micronuclei was seen following oral dosing up to a lethal dose (80 mg/kg) to CD-1(ICR) mice. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data (MRID No. 44651852).
- (6) In an *in vivo/in vitro* unscheduled DNA synthesis assay, acetamiprid at concentrations ranging from 75 mg/kg to 300 mg/kg did not induce UDS in primary rat hepatocytes. However, this study is classified as unacceptable since no toxicity was induced at the HDT, and an insufficient number of rats was used at the harvest times (MRID No. 44651853)
- (7) In repeat assays for *in vitro* unscheduled DNA synthesis, when tested in liver primary cells cultures from adult male Fischer 344 rats, acetamiprid did not induce UDS. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guidelines 84-2 for other genotoxic mutagenicity data (MRID No. 44651856).

Studies on Metabolites

IM-0

In repeat reverse gene mutation assays in *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2 uvrA) at concentrations of test material ranging from 313 to 5000 µg/plate, both in the presence and absence of metabolic activation, IM-0 did not increase the number of revertants at any dosage. Positive controls induced significant increases in the number of revertants. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) (MRID No. 44988432).

IM-1-2

In repeat reverse gene mutation assays in *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2 uvrA) at concentrations of test material ranging from 313 to 5000 µg/plate, both in the presence and absence of metabolic activation, IM-1-2 did not increase the number of revertants at any level. Positive controls induced significant increases in the number of revertants. There was no demonstrable evidence of cytotoxicity or precipitation. This study is classified as acceptable and does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) (MRID No. 44651850).

In repeat reverse gene mutation assays in *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2 uvrA) at concentrations of test material up to 5000 µg/mL, both in the presence and absence of metabolic activation, IM-1-2 did not increase the number of revertants at any dosage. Positive controls induced significant increases in the number of revertants. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) (MRID No. 44988433).

IM-1-4

IM-1-4 was tested in repeat reverse gene mutation assays in *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* (WP2 uvrA) at concentrations from 313 to 5000 µg/plate, both in the presence and absence of metabolic activation. In the absence of metabolic activation, there were no increases in the number of revertants in any strains. Although the number of revertants increased in the presence of metabolic activation, this increase was within two-fold of the vehicle control. Growth inhibition was observed at 5000 µg/plate in the absence of metabolic activation, as well as in three *Salmonella* strains (except TA1535) in the presence of metabolic activation. Since no concentration-response relationship nor reproducibility was observed, IM-1-4 was considered negative under these experimental conditions by the investigators. This study is classified as acceptable and does satisfy the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene

mutation) data in its present form (MRID No. 44651851).

IM-1-4 was tested in a Chinese Hamster Ovary Cell HGPRT mammalian cell gene mutation assay at doses ranging from 250 to 5000 µg/mL, both in the presence and absence of mammalian metabolic activation. Severe toxicity was observed at 3000 µg/mL -S9 and at 3500 µg/mL +S9. IM-1-4 is evaluated as negative for inducing forward mutations at the HGPRT locus in CHO cells under both S9 metabolic activation and nonactivation conditions of the assay. This study is classified as acceptable, and satisfies the FIFRA Test Guideline requirement for *in vitro* mammalian cell mutation data (MRID No. 44988431).

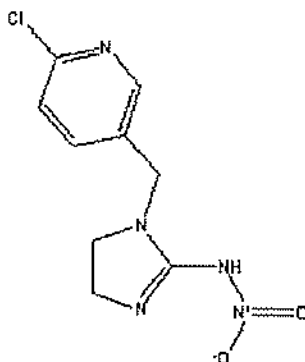
In an *in vivo* micronucleus study, IM-1-4 was tested up to clinical toxicity (700 mg/kg) to the treated animals and cytotoxicity (≥ 350 mg/kg) to the bone marrow (statistically decreased in the PCE:NCE ratio). A statistically significant increase in micronucleated polychromatic erythrocytes (MPE) was induced, but only in 350 mg/kg females at the 24-hour harvest time point; however, the response was not dose- or time-related and was within the range of historical vehicle controls, and thus is not considered as biologically relevant. The positive control, cyclophosphamide induced a significant increase in MPEs at the 24-hour harvest in both males and females. Therefore IM-1-4 is considered negative in the mouse bone marrow micronucleus test under the conditions of exposure in this assay. This study is classified as acceptable and satisfies the FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data (MRID No. 44988501).

IC-0

In repeat reverse gene mutation assays in *Salmonella typhimurium* (TA100, TA1535, TA98 and TA1537) and *Escherichia coli* (WP2 uvrA) at concentrations of test material up to 5000 µg/plate (level of growth inhibition and/or precipitation with S9), both in the presence and absence of metabolic activation, IC-0 did not increase the number of revertants at any dosage. Positive controls induced significant increases in the number of revertants. This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) (MRID No. 44988502).

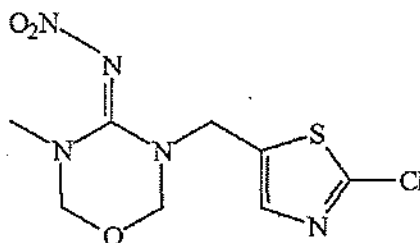
3. Structure-Activity Relationship

Acetamiprid (PC Code 099050; CAS No. 135410-20-7) belongs to a new class of compounds, the neonicotinoids. Related compounds are identified in three subclasses of the neonicotinoids. Acetamiprid is in the chloronicotinyl subclass, which is the same subclass as imidacloprid, and nitenpyram. The Agency has no data on nitenpyram. Thiamethoxam is also in the neonicotinoid class; however, it is in a different subclass than acetamiprid.



Imidacloprid

Imidacloprid (PC Code 129099; CAS No. 138261-41-3) tested negatively in rat and mouse carcinogenicity bioassays. It also tested negatively in all of the mutagenicity studies except for the *in vitro* chromosomal aberration study, where it tested positively at 500 $\mu\text{g/mL}$ (without S9) and 1300 $\mu\text{g/mL}$ (with S9); however, both doses were considered to be toxic. It has been classified by the HED Peer Review/Cancer Committee (cf. Nov 10, 1993 Report) as a Group E carcinogen based on the following evidence: no apparent treatment-related carcinogenic effect at any dose in a chronic/carcinogenicity feeding study in the rat (MRID #422563-31, 422563-32) or in a carcinogenicity study in the mouse (MRID #422563-35, 422563-36).



Thiamethoxam

Thiamethoxam (PC Code 060109; CAS No. 153719-23-4) was classified as "likely to be carcinogenic to humans" by the oral route based on the occurrence of both benign and malignant hepatocellular tumors in both sexes of mice. Although no tumors were observed in rats, a hepatocarcinogenic response cannot be ruled out because the animals were not tested at higher dose levels. The CARC recommended a linear low-dose extrapolation approach for the quantification of human cancer risk based on the most potent of the liver tumor response observed in mice. This approach is supported by the lack of confirmation of the mode of action of

thiamethoxam. (CARC, April 5, 2000). Thiamethoxam was negative in both *in vitro* and *in vivo* mutagenicity assays. Mechanistic data were available for thiamethoxam; however, the Mechanism of Toxicity Assessment Review Committee (MTARC) concluded that the available data were insufficient to support the proposed non-linear mode of action for liver carcinogenicity of thiamethoxam. The Q1* for thiamethoxam is 3.77×10^{-2} human equivalents.

4. Subchronic and Chronic Toxicity

a) Subchronic Toxicity

Mice

In a subchronic oral toxicity study (MRID 44988425), groups of Crl:CD-1™ (ICR) mice (10 mice/sex/group) were administered acetamiprid 0, 400, 800, 1600, or 3200 ppm of 31-1359 (Lot No. 591001-7; 99.2% a.i.) in the diet for at least 90 days. Time-weighted average doses were 0, 53.2, 106.1, 211.1, and 430.4 mg/kg/day, respectively, for males and 0, 64.6, 129.4, 249.1, and 466.3 mg/kg/day, respectively, for females.

The results of this study are discussed in the adequacy of dosing section for the mouse carcinogenicity study. **The LOAEL for male and female mice is 1600 ppm (211.1 and 249.1 mg/kg/day, respectively) based on reduced body weights and body weight gains. The NOAEL for males and females is 800 ppm (106.1 and 129.4 mg/kg/day, respectively).**

Rats

1) In a subchronic oral toxicity study (MRID 44651843), 31-1359 (>99% a.i.; lot number:31-0223-HY [Tox-447]) was administered to groups of 10 Crl:CD (Sprague-Dawley) rats/sex/dose in the diet at dose levels of 0, 50, 100, 200, 800, or 1600 ppm (0, 3.1, 6.0, 12.4, 50.8, and 99.9 mg/kg/day for males, respectively, and 0, 3.7, 7.2, 14.6, 56.0, and 117.1 mg/kg/day for females, respectively) for 13 weeks.

The results of this study are discussed in the adequacy of dosing section for the combined chronic feeding/oncogenicity study in the rat. **The LOAEL for male and female rats is 800 ppm (50.8 and 56.0 mg/kg/day, respectively) based on dose-related decreases in body weights, body weight gains, and food consumption. The NOAEL for male and female rats is 200 ppm (12.4 and 14.6 mg/kg/day, respectively).**

2) In a subchronic neurotoxicity study (MRID #44651845), groups of fasted, male and female Crl:CD-BR rats (10/sex/dose), were given daily doses of acetamiprid (99.9%) in the diet for 90 days at doses of 0, 100, 200, 800 and 1600 ppm (equal to 0, 7.4, 14.8, 59.7 and 118 mg/kg bw/day for males and 0, 8.5, 16.3, 67.6, and 134 mg/kg bw/day for females).

There were no mortalities or clinical signs of toxicity recorded during the course of the study. Treatment with acetamiprid had no effect on brain weight, motor activity, behavior or neuropathology. Body weights, body weight gain, food consumption and food efficiency were reduced in male and female rats at 800 and 1600 ppm.

The LOAEL was 800 ppm (equal to 59.7 and 67.6 mg/kg bw/day for males and females respectively) based on reductions in body weight, body weight gain, food consumption and food efficiency. The NOAEL was 200 ppm (equal to 14.8 and 16.3 mg/kg bw/day for males and females respectively).

Dogs

1) In a subchronic toxicity study (MRID 44988424), acetamiprid (99.46% a.i.) was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 320, 800 and 2000 ppm (equal to 0, 13, 32 and 58 mg/kg bw/day in males and 0, 14, 32 and 64 mg/kg bw/day in females) for 90 days.

Treatment with acetamiprid had no effect on mortality, clinical signs of toxicity, ophthalmoscopic examinations, hematology, clinical chemistry, urinalysis, organ weights and macroscopic or microscopic pathology. Group mean body weight and body weight gain was significantly reduced among high dose males and females (animals at this dose lost weight over the course of the study). Decreased body weight gain was observed in males and females at 800 ppm during the first few weeks of the study, such that total gain over the study period was 29% of control in males and 67% of control in females. Decreases in food consumption were consistent with the observed changes in body weight and body weight gain.

The LOAEL was 800 ppm (equal to 32 mg/kg bw/day in males and females), based on the observed reduction in body weight gain in animals of both sexes. The NOAEL was 320 ppm (equal to 13 mg/kg bw/day in males and 14 mg/kg bw/day in females).

2) In a subchronic toxicity study (MRID 45245306), acetamiprid (99.46% a.i.) was administered to 2 Beagle dogs/sex/dose in the diet at dose levels of 0, 125/3000, 250, 500 and 1000 ppm (equal to 0, 4.1/42.5, 8.4, 16.7 and 28.0 mg/kg bw/day in males and 0, 4.8/46.2, 8.7, 19.1 and 35.8 mg/kg bw/day in females) for 28 days.

Treatment with acetamiprid had no effect on mortality, clinical signs of toxicity, hematology, clinical chemistry and macroscopic pathology. After two weeks of treatment, the 125 ppm group dose was increased to 3000 ppm and continued for 4 weeks. Upon initiation of dosing at 3000 ppm, a marked decrease in food consumption was observed. Significant body weight loss was observed at 3000 ppm, and a decrease in body weight gain was observed at 1000 ppm. Slightly reduced absolute and relative (to brain) kidney and liver weights were observed among 3000 ppm animals, which were considered to reflect the observed changes in body weight at that dose.

The LOAEL was 1000 ppm (equal to 28.0 and 35.8 mg/kg bw/day in males and females, respectively), based on the observed reduction in body weight gain in animals of both sexes.

The NOAEL was 500 ppm.

b) Chronic Toxicity

Dogs

In a 1-year toxicity study (MRID 44651846), acetamiprid (99.57% a.i.) was administered to 4 Beagle dogs/sex/dose in the diet at dose levels of 0, 240, 600 and 1500 ppm (equal to 0, 9, 20 and 55 mg/kg bw/day in males and 0, 9, 21 and 61 mg/kg bw/day in females) for 1 year.

Treatment with acetamiprid had no effect on mortality, clinical signs of toxicity, ophthalmology, hematology, clinical chemistry, urinalysis and gross or microscopic pathology. Decreased body weight, body weight gain and food consumption were recorded in high-dose male and female animals. There were no effects of treatment on absolute organ weights nor organ-to-body weight ratios. Significantly decreased kidney-to-brain weight and liver-to-brain weight ratios were attributed to the significant reductions in body weight observed at that dose.

The LOAEL was 1500 ppm (equal to 55 and 61 mg/kg bw/day in males and females, respectively), based on the initial body weight loss and overall reduction in body weight gain in animals of both sexes. The NOAEL was 600 ppm (equal to 20 and 21 mg/kg bw/day in males and females, respectively).

Rats and Mice

The chronic rat and mouse studies conducted with acetamiprid are discussed previously in the Evaluation of Carcinogenicity Studies section.

5. Mode of Action Studies

There are no mode of action studies available at this time.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that acetamiprid was not carcinogenic to male and female rats and mice based on the following:

- Although male rats had a significant increasing trend ($p < 0.05$) in interstitial cell tumors of the testes, there were no significant differences in the pair-wise comparisons of the dosed groups with the controls. The incidence at the high dose (1000 ppm; 10 % versus 2% in controls) was within the range of the historical controls for Charles River Laboratories (1.4%-10%). The CARC considered that testicular tumors are common to this strain

of rat and concluded that the increase in these tumors was not treatment-related.

- For female rats, there were significant differences ($p < 0.05$) in the pair wise comparisons of the 400 ppm dose group with the controls for pituitary adenomas, and combined adenomas/adenocarcinomas but increases in these tumors were not seen at the high dose (1000 ppm). The incidences at 400 and 1000 ppm were as follows: adenomas: 48/59, 81% and 44/60, 73% vs 38/60, 63% in controls; combined adenomas/adenocarcinomas: 50/59, 85% and 46/60, 77% vs 39/60, 65%, respectively). These incidences did not exceed the available historical control range for this tumor. **The increased pituitary tumor incidence was not dose-related and, therefore, was not considered by the CARC to be treatment-related.**
- Female rats had a significant increasing trend in adenocarcinomas (borderline; $p = 0.05$) and combined mammary gland adenomas/adenocarcinomas ($p < 0.05$) but no significant differences were seen in pair-wise comparisons of the dosed groups with the controls, for these tumors. The incidences of mammary gland adenomas at the mid- and high dose groups (400 and 1000 ppm, respectively; adenomas: 4/59, 7% and 3/60, 5% vs 1/59, 2% in controls; and combined adenomas/adenocarcinomas: 17/59, 29% and 19/60, 32% vs 11/59, 19% in controls, respectively) were within the historical control range for Charles River Laboratories (2%-32% and 8.6%-58.3%, respectively). Although the incidence of adenocarcinomas at mid- and high-dose groups (27% and 28%, respectively) exceeded the historical control range of the testing laboratory (14%-18%; mean: 16%), it was within the range observed in Charles River historical control data (7-31%) and did not reach statistical significance by pair wise comparison with the controls. The combined incidence was driven by adenocarcinomas. Although there was an increase in the incidence of mammary adenocarcinomas in the mid- and high-dose groups, an increased incidence of mammary tissue hyperplasia was seen only in high-dose females; there was no increase in severity and there was no increase in the incidence of galactocoele in the mammary tissue. **The Committee, therefore, concluded that these tumors were not treatment-related.** The dosing at the highest level was considered to be adequate and not excessive based on decrease in body weight gain in males (18%) and females (23%); decrease in food consumption in both sexes as well as renal changes and liver hypertrophy and hepatocyte vacuolation in males.
- There was no treatment-related increase in tumors in male and female mice. Dosing at the highest level (1200 ppm) was considered by the CARC to be adequate and not excessive, based on a decreases in body weight gain in males and females (12% and 11%, respectively at 13 weeks and 55% and 58%, respectively, at 78 weeks), liver hypertrophy in high dose males and females as well as myeloid hyperplasia in mid- and high dose males and high dose females at 78 weeks.
- Acetamiprid was clastogenic in the Chinese Hamster Ovary cell assay. However, both acetamiprid and its metabolites, IM-1-4, IM-1-2, IM-0 and IC-0, were not mutagenic in *in vitro* and/or *in vivo* assays. The available studies satisfy pre-1991 FIFRA guideline

requirements. At this time, the Committee has no concern for mutagenicity of acetamiprid.

- The structurally-related compound, imidacloprid was not mutagenic in all mutagenicity studies except for the *in vitro* chromosomal aberration assay. The positive response was, however, only noted at cytotoxic doses. It was not carcinogenic in rats and mice and, therefore, was classified as category "E" (not carcinogenic to humans). A second more distantly related compound, thiamethoxam, was not mutagenic in *in vitro* and *in vivo* mutagenicity assays. However, it has been classified as "Likely to be carcinogenic to humans by the oral route based on an increased incidence of liver tumors in male and female mice." The hepatocarcinogenic response for thiamethoxam in rats could not be ruled out because the animals were not tested at adequate dose levels.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

The majority of members concluded that acetamiprid was not likely to be carcinogenic to humans because: there was 1) an absence of a dose-response and a lack of a statistically significant increase in the mammary adenocarcinoma incidence by pair wise comparison of the mid- and high-dose groups with the controls; although the incidence exceeded the historical control data from the same lab, it was within the range of values from the supplier; also the control incidences in this study were on the high side; 2) a lack of an increase in the incidence of galactocoele in the mammary tissue; and 3) an increased incidence of mammary tissue hyperplasia noted in high-dose females but not in mid-dose females.

Three members believed that the mammary tumor response, although marginal, cannot be discounted because: 1) there was an increase in the incidence of adenocarcinomas at the two top doses and the incidence exceeded the historical control range based on three studies from the testing laboratory and 2) the combined incidence of mammary adenomas/adenocarcinomas was driven by adenocarcinomas (see attachment for rationale supporting classification as "Suggestive..."). One member felt that the data were inadequate to assess the carcinogenic potential of acetamiprid because: 1) the appropriate historical control data were not available for relevant comparison, and 2) the animals could have tolerated a higher dose.

Based on the majority opinion and in accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified acetamiprid into the category "Not likely to be carcinogenic to humans".

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of human cancer risk is not required.

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Subject: Minority Opinion on Acetamiprid Classification

From: Esther Rinde, Ph.D.

To: William Burnam, Chair
Cancer Assessment Review Committee (CARC)

Administration of Acetamiprid in the diet was associated with an increased incidence of mammary gland adenocarcinomas in the female Crl:CD/BR rat. While the increased incidence of adenocarcinomas (malignant) was not statistically significant in comparison with concurrent controls, the incidences of the mid- and high-dose groups exceeded that of historical controls for the testing facility and there was a borderline statistically significant positive trend. Although this may be considered only a marginal increase, I do not feel it can be dismissed.

As I stated at the 10/31/2001 CARC meeting, I believe that in accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the classification for Acetamiprid should be: **"Suggestive evidence of carcinogenicity but not Sufficient to assess Human Carcinogenic Potential"** According to these Guidelines: "This descriptor is appropriate when the evidence from human or animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is judged not sufficient for a conclusion as to human carcinogenic potential. Examples of such evidence may include: *a marginal increase in tumors that may be exposure-related*, or evidence is observed only in a single study, or the only evidence is limited to certain high background tumors in one sex of one species. Dose-response assessment is not indicated for these agents." I believe this to be a better descriptor for the mammary gland tumors in the female rat than that of the majority of the CARC ("Not Likely..") - which requires that "...data are considered robust for deciding that there is no basis for human hazard concern..".

Acetamiprid - Crl:CD@BR Rat Study: Female Mammary Gland Tumor Rates[†] and Exact Trend Test and Fisher's Exact Test Results (Brunsmann, 2001)

	0 ppm	160 ppm	400 ppm	1000 ppm	Historical Control	
					Range (%)	Mean (%)
Adenomas (%) p =	1/59 (2) 0.0888	0/60 (0) 0.4958	4/59 (7) 0.1821	3 ^a /60 (5) 0.3157	2-32 ^f	8
Adeno-carcinomas (%) p =	10/59 (17) 0.0543	11 ^b /60 (18) 0.5171	16/59 (27) 0.1333	17/60 (28) 0.1029	14-18 ^c 9-58 ^f	16 24
Combined (%) p =	11/59 (19) 0.0294*	11/60 (18) 0.5760	17 ^c /59 (29) 0.1396	19 ^d /60 (32) 0.0768	8.6- 58.3% ^f	

[†] Number of tumor bearing animals/Number of animals examined, excluding those that died before week 31.

^a First adenoma observed at week 53, dose 1000 ppm.

^b First adenocarcinoma observed at week 31, dose 160 ppm.

^c Three animals in the 400 ppm dose group had both an adenoma and an adenocarcinoma.

^d One animal in the 1000 ppm dose group had both an adenoma and an adenocarcinoma.

^e Historical control data from same testing laboratory ^f Historical control data from Charles River Laboratories (published in 1992). Note: Significance of trend denoted at control; Significance of pair-wise comparison with control denoted at dose level: If *, then p<0.05.

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