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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

TXR NO. 0050279

DATE: November 20, 2001

MEMORANDUM

SUBJECT: ***ACETAMIPRID*** - Report of the Hazard Identification Assessment Review Committee.

FROM: Pamela M. Hurley, Toxicologist.
Registration Action Branch 2
Health Effects Division (7509C)

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

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

PC Code: 099050

On September 20, 2001 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Acetamiprid with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Acetamiprid was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.

Committee Members in Attendance

Members present were: Ayaad Assaad, William Burnam, Paula Deschamp, Elizabeth Doyle, Pamela Hurley, John Liccione, Susan Makris, David Nixon, Jess Rowland, Brenda Tarplee

Member(s) in absentia: Jonathan Chen

Data evaluation prepared by: Pamela Hurley, Registration Action Branch 2
Gordon Cockell, PMRA Canada

Also in attendance were:

Data Evaluation / Report Presentation

Pamela Hurley
Toxicologist

1. INTRODUCTION

On September 20, 2001 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for Acetamiprid with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for use as appropriate in occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to Acetamiprid was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD) - General Population, Including Infants and Children

Study Selected: Acute neurotoxicity study - rat § 870.6200

MRID No.: 44651841

Executive Summary: In an acute neurotoxicity study (MRID # 44651842), groups of fasted, male and female Crl:CD-BR rats (10/sex/dose), were given a single oral dose of Acetamiprid (99.9%) by gavage, in 0.5% sodium carboxymethylcellulose at doses of 0, 10, 30, or 100 mg/kg bw and observed for 14 days. There were no mortalities during the study. Body weight gain and food consumption were significantly reduced in high-dose males. Body weight, body weight gain, food consumption and food efficiency were unaffected in females. Treatment with acetamiprid had no effect on brain size or weight and there was no evidence of neuropathology. Clinical signs of toxicity were limited to the high-dose animals, and included tremors, hunched posture, unsteady gait and coldness to touch. In addition, one high-dose female had slight brown nasal staining from study day 2 until termination.

High-dose males and females had significantly reduced body temperature on the day of dosing. Significantly decreased motor activity was observed in mid- and high-dose males and in high-dose females on the day of dosing. A slight decrease in the duration of movements persisted in mid- and high-dose males on days 7 and 14. Functional observational battery evaluations revealed several treatment-related observations on the day of dosing. High-dose males exhibited tremors, difficulty in handling, walking on toes, dilated pupils and coldness to the touch. High-dose males also had decreased forelimb grip strength and hind limb foot splay. High-dose females displayed tremors, chewing, coldness to the touch and dilated pupils. High-dose females had decreased hind limb foot splay. High-dose females were seen to have abnormal gaits and/or posture, including walking on toes and hunched posture.

The LOAEL for neurotoxicity was 30mg/kg bw, based on the observed reduction in locomotor activity in males. **The NOAEL for neurotoxicity was 10mg/kg.**

This study is classified acceptable, and satisfies the guideline requirement for an acute neurotoxicity study (870.6200; OECD 424) in the rat.

Dose and Endpoint for Establishing RfD: 10 mg/kg based on decreased motor activity at the LOAEL of 30 mg/kg.

Uncertainty Factor (UF): 100

Comments about Study/Endpoint/Uncertainty Factor: The route and duration of exposure are appropriate for selection of the acute dietary endpoint; effects were observed after a single oral dose.

Acute RfD = $\frac{\text{10 mg/kg (NOAEL)}}{\text{100 (UF)}}$ = **0.10 mg/kg**

2.2 Chronic Reference Dose (RfD)

Study Selected: Chronic/Oncogenicity Study in the Rat § 870.4300

MRID Nos.: 44988429, 45245304

Executive Summary: In a chronic toxicity/oncogenicity study (MRID 44988429 & 45245304), 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.

There were no treatment-related effects on mortality; eyes; hematology, clinical chemistry or urinalysis parameters; or gross findings in either sex administered any dose of the test material. Clinical signs that were observed at significantly increased incidences in treated animals included rales in high dose males (7/48 vs 0/46 for controls) during weeks 66-78 and at all doses in males during weeks 79-91 (0/44, 8/49, 19/45, and 17/48 at 0, 160, 400, and 1000 ppm, respectively). Also in high-dose male rats, the incidences of labored breathing (15/48 vs 5/46 for controls, $p<0.05$) was increased during weeks 66-78, red material around the nose during weeks 1-13 (7/60 vs 0/60 for controls) and weeks 92-104 (5/46 vs 0/37), and hunched posture (5/46 vs 0/37) during weeks 92/104. The lack of pathologic correlates indicate that the clinical signs are not biologically significant.

Treatment-related effects on body weight, body weight gain, and food consumption were observed in both sexes. High-dose male rats weighed 10-13% ($p<0.01$) less than controls

throughout the study, gained 44% less weight during week 1, 14% less during the first year and 18% less over the entire study. High-dose group males also consumed 19% ($p<0.01$) less food (g/animal/day) during week 1 and 4-9% ($p<0.01$ or <0.05) less at different time points during the remaining weeks of the study. Food efficiency measured during the first 14 weeks was reduced for males in all dose groups during the first week of the study and showed an inconsistent pattern for the remaining 13 weeks. Mid-dose female rats weighed 4-17% ($p<0.01$) less than controls throughout the study and high-dose females weighed 6-27% ($p<0.01$) less. Mid- and high-dose group females, respectively, gained 27 and 42% less weight than controls during week 1, 15% and 32% less during the first year, and 16% and 23% less over the entire study. Food consumption was 6-10% and 9-19% less for mid- and high-dose group females, respectively, for most of the study. Food efficiency was reduced for mid- and high-dose group females during week 1 and showed inconsistent patterns for the remaining 13 weeks.

The postmortem examination showed statistically significant changes in absolute and/or relative weights of several organs in high-dose group male and female rats, and these changes are attributed to the decreased terminal body weight. Treatment-related microscopic changes were observed in the liver, kidney, and mammary glands. Trace to mild hepatocyte hypertrophy in the liver of mid- and high-dose male rats and high-dose group female rats at interim sacrifice and in the main study groups is considered an adaptive response rather than an adverse effect. Hepatocyte vacuolation also was observed in mid- and high-dose group male rats; the incidence was 10/12 and 10/11, respectively, compared with 2/12 for controls at interim sacrifice and 22/48 and 29/48, respectively, compared with 10/48 for controls in the main study. An increased incidence of microconcretions in the kidney papilla was noted for high-dose male rats (37/49 vs 17/48 for controls, $p<0.01$) in the main study. The incidence of 24/49 ($p<0.05$) for mammary hyperplasia in high-dose group females compared with 14/49 for controls appeared to be treatment related, but the toxicologic significance of this finding is uncertain.

The lowest-observed-adverse-effect (LOAEL) for NI-25 is 400 ppm (17.5 mg/kg/day for males and 22.6 mg/kg/day for females) for male and female rats based on reduced body weight and body weight gain for females and hepatocellular vacuolation for males. The no-observed-adverse-effect level (NOAEL) is 160 ppm (7.1 mg/kg/day for males and 8.8 mg/kg/day for females)

At the doses tested, there was some evidence of a treatment-related increase in tumor incidence when compared to controls. The incidence of mammary adenocarcinoma was significantly increased in females (9/49, 10/49, 15/47 (32%), and 17/49 (35%, $p<0.05$) for 0, 160, 400, and 1000 ppm, respectively) . The incidence of 32% at the mid dose and 35% at the high dose exceeded that of historical controls at the testing laboratory, MPI (13.3-28.6%), but was within range of historical controls for Charles River Laboratories (0-37.2%). Dosing was considered adequate based on significantly decreased mean body weight gain when compared to the control groups in both sexes and an increased incidence of hepatocyte vacuolation in male rats.

This chronic toxicity /oncogenicity study in the rat is **Acceptable/Guideline** and satisfies the

guideline requirements for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. No deficiencies were noted for this study.

Dose and Endpoint for Establishing RfD: 7.1 mg/kg/day based on decrease in mean body weight, body weight gain and hepatocellular vacuolation at the LOAEL of 17.5 mg/kg/day.

Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor: The route and duration of exposure are appropriate for selection of the chronic dietary endpoint.

$$\textbf{Chronic RfD} = \frac{\text{7.1 mg/kg/day (NOAEL)}}{\text{100 (UF)}} = \textbf{0.07 mg/kg/day}$$

2.3 Occupational/Residential Exposure

2.3.1 Short- (1 Day - 1 Month) and Intermediate-Term (1 Month - 6 Month) Incidental Oral Exposure

Studies Selected:	13-Week Rat	§ 870.3100
	Subchronic mammalian neurotoxicity	§ 870.6200
	Developmental rat	§ 870.3700

MRID Nos.: 44651843
44651845
44651847

Executive Summaries: 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 Crj: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.

Treatment with 31-1359 induced a dose-related reduction of growth rate in males and females as indicated by decreases in body weights, food consumption, food efficiency, and/or absolute organ weights.

In animals fed 800 ppm 31-1359, decreases in mean absolute body weights were observed in males from weeks 1-12 (90-92% of controls; $p<0.05$; 0.01 except week 11) and in females during weeks 6-13 (89-90%; statistically significant at weeks 6-8; $p<0.05$). During the treatment period, 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 in 800-ppm males at week 1 (80% of controls; $p<0.01$) and in 800 ppm females at weeks 1-7, 10, 12, and 13 (80-91% of controls; statistically significant at weeks 2 and 3: $p<0.05$; 0.01). No statistically significant differences were observed in mean food efficiencies.

In animals fed 1600 ppm 31-1359, males and females had decreases in mean absolute body weights at each week of treatment (85-87%; $p<0.05$; 0.01 for males; 77-90%; $p<0.01$ for females), with final mean absolute body weights being 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 high-dose males during weeks 1-7 (78-91% of controls; significant at weeks 1, 2, and 7; $p<0.01$), and in high-dose females during weeks 1-13 (73-91% of controls; significant at weeks 1-7 and 11; $p<0.05$; 0.01). Mean food efficiency was statistically ($p<0.05$; 0.01) decreased in high-dose males at weeks 1 and 6 (52 and 79% of controls, respectively), and in high-dose females at weeks 1, 3, and 6 (41, 66, and 47% of controls, respectively). High-dose females additionally had changes in organ weights consistent with reduced body weights, including decreased ($p<0.05$; 0.01) absolute weights of heart (87%), kidneys (87-90%), and adrenals (79-80%), and increased relative weights of brain (126%), lung (123%), heart (113%), and kidneys (112-116%).

Increased levels of total cholesterol were observed in high-dose males (141% of controls; $p<0.01$) and females (124% of controls, n.s.). Liver weights relative to body weights were increased ($p<0.05$; 0.01) in 800 and 1600 ppm males (113 and 126% of controls, respectively) and females (115 and 128% of controls, respectively). 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.

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).

This subchronic oral toxicity study in the rat is **Acceptable/Guideline** and satisfies the requirements for a subchronic oral toxicity study [OPPTS 870.3100 (§82-1a)] in rats.

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, behaviour 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).**

This study is classified acceptable, and satisfies the guideline requirement for a subchronic neurotoxicity oral study in the rat.

In a **developmental toxicity study (MRID 44651847)**, acetamiprid (99.46% a.i.) was administered to 24 female Crj:CD (SD) rats/dose in 5% arabic gum and 0.01% Tween 80 in water, by gavage at dose levels of 0, 5, 16 or 50 mg/kg bw/day from days 6 through 15 of gestation.

There was no mortality, nor were there any clinical signs of toxicity noted in the study. Treatment with acetamiprid did not affect gross pathology nor cesarean section parameters. Maternal body weight, body weight gain and food consumption were reduced at 50 mg/kg bw/day, and absolute and relative liver weights were increased at 50 mg/kg bw/day. **The maternal LOAEL is 50 mg/kg bw/day, based on the observed reductions in body weight, body weight gain and food consumption and increased liver weights. The maternal NOAEL is 16 mg/kg bw/day.**

Treatment with acetamiprid did not affect the number of fetuses, fetal sex ratios or fetal weights. There were no treatment related changes in fetal external nor visceral examinations. There was an increase in the incidence of the skeletal variation, shortening of the 13th rib, at 50 mg/kg bw/day. **The developmental LOAEL is 50 mg/kg bw/day, based on the increased incidence of shortening of the 13th rib. The developmental NOAEL is 16 mg/kg bw/day.**

This developmental toxicity study in the rat is classified acceptable, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

Dose and Endpoint for Risk Assessment: 15 mg/kg/day based on decreased body weight, body weight gain and food consumption in three co-critical studies at the lowest LOAEL of the three studies of 50 mg/kg/day.

Comments about Studies/Endpoint: There are three co-critical studies, the subchronic feeding study in the rat, the subchronic mammalian neurotoxicity study and the developmental toxicity study in the rat. All three studies had similar endpoints: decreases in body weight, body weight gain and food consumption. One study, the subchronic neurotoxicity study noted a reduction in food efficiency as well. The NOAEL's for the three studies are 12.4, 14.8 and 16 mg/kg/day for the subchronic feeding, subchronic neurotoxicity and developmental toxicity studies, respectively. There are 4-week measurements for these effects in the 13-week feeding study in the rat with the same NOAEL/LOAEL, which will cover short-term exposure. The effects are also considered to be sustained effects because they are observed over several time points, thus covering intermediate-term exposure. Lastly, it is noted that the NOAEL selected for this endpoint is higher than the NOAEL selected for the acute dietary endpoint. This is likely a result of dose selection. The acute neurotoxicity study, upon which the acute dietary endpoint is based had a NOAEL of 10 mg/kg and a LOAEL of 30 mg/kg. The NOAELs/LOAELs for the subchronic feeding, subchronic neurotoxicity and developmental toxicity studies, respectively are 12.4/50.8, 14.8/59.7 and 16/50 mg/kg/day, respectively. The endpoint is appropriate for the population (infants and children) and exposure durations of concern.

2.3.2 Dermal Absorption

Dermal Absorption Factor: 30% based on a dermal absorption study in the rat with a conservative estimate at 24 hours.

Study: Dermal Absorption Study in Rats § 870.7600

MRID No.: 44651858

Executive Summary: The dermal absorption of NI-25 (Acetamiprid) was determined in male rats at doses of 1.09, 9.53 and 90.2 ug/cm^2. Exposure durations were 0.5, 1, 2, 4, 10 and 24 hours, four rats per dose duration. Recovery at all doses was good ranging from 96.6 to 102 % of dose. The majority of the dose was washed off with the percent increasing with dose (63.6-75.8, 64.9-78.8 and 79.3-87.5 respectively). Skin residue was the next largest portion of the dose with the percent decreasing with dose (21.7-29.1, 20.8-26.5 and 10.2-16.9 respectively). In neither case was there evidence of an exposure related pattern.

Absorption of the definitive study was as follows. Absorbed is defined as the sum of blood, carcass, cage wash, cage wipe, urine and feces.

Exposure	13.6 ug/rat 1.09 ug/cm²			119 ug/rat 9.53 ug/cm²			1,130 ug/rat 90.2 ug/cm²		
(hours)	%	ug/rat	ug/cm²	%	ug/rat	ug/cm²	%	ug/rat	ug/cm²
0.5	NC	NA	NA	0.16	0.190	0.015	0.34	3.84	0.307
1	0.33	0.045	0.004	0.63	0.750	0.060	0.16	1.81	0.144
2	0.33	0.045	0.004	0.45	0.536	0.043	0.27	3.04	0.244
4	1.20	0.163	0.013	1.02	1.21	0.115	0.64	7.23	0.577
10	1.48	0.201	0.016	4.07	4.84	0.388	0.78	8.81	0.704
24	4.27	0.581	0.047	6.34	7.54	0.604	2.82	31.9	2.54

NC not calculated. Two or more individual values were Not Detectable and/or <0.005%
NA Not Applicable

Absorption was small and increased with duration of exposure. The quantity absorbed increased with dose but the percent absorbed increased between the low and intermediate doses and decreased between the intermediate and high doses. This is an unusual pattern. Since there are no data to demonstrate that the residues remaining on the skin do not enter the animal, then as a conservative estimate of dermal absorption, residues remaining on the skin will be added to the highest dermal absorption value (6.34% at 24 hours). The residue remaining on the skin at 24 hours is 25.0% of the dose (9.53 µg/cm²). Therefore, the potential total absorption at 24 hours could be 25.0 + 6.34 or approximately 30%.

2.3.3 Short- (1 Day - 1 Month) and Intermediate-Term Dermal (1 Month - 6 Month) Exposure

Study Selected: 2-Generation Reproduction Study in the Rat § 870.3800

MRID No.: 44988430

Executive Summary: In a two-generation reproduction study (one litter per generation, MRID 44988430) Acetamiprid (99.9% a.i.) was administered to 26 Crl:CD BR (IGS) Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 100, 280, or 800 ppm (equal to 0, 6.5, 17.9 or 51.0 and 0, 7.6, 21.7 or 60.1 mg/kg bw/day in males and females, respectively).

There were no treatment-related mortalities or clinical signs of toxicity among parental animals in either generation. In addition, there were no definitive treatment-related clinical signs among F_1 or F_2 pups. In the F_1 parental generation, two 100 ppm females and five 800 ppm dams experienced total litter death. There was an equivocal association with the incidence of thin, pale and/or weak pups among those litters that experienced total litter death, such that the combined incidence of those clinical signs suggested a possible relationship to treatment with acetamiprid. Mean litter size (day 4 pre-cull), viability index and weaning index were significantly reduced at 800 ppm among F_2 pups. Mean litter size was also reduced among F_1 pups on lactation days 14 and 21.

Body weight, body weight gain and food consumption were reduced during the premating period among males and females at 800 ppm in both generations. A slight, transient, non-adverse reduction in body weight gain and food consumption was observed in males of both generations at 280 ppm for the first few weeks (2-5) on the test diets. Maternal body weight and body weight gain were also reduced during the gestation period, however body weight gain tended to increase during the lactation period at 800 ppm.

There were no treatment-related changes in reproductive function tests, including estrous cycle length and periodicity and sperm motility, count and morphology. Similarly, there were no treatment-related changes in reproductive performance in either generation. Decreases in absolute and relative organ weights at 800 ppm were attributed to the observed reduction in body weight among these animals. There were no treatment-related macroscopic or microscopic pathology findings in this study.

In addition to the litter size, viability index and weaning index observations noted among offspring, significantly reduced pup weights were observed throughout the lactation period in males and females of both generations at 800 ppm. The mean age to attain vaginal opening was significantly increased for females at 800 ppm and the mean age to attain preputial separation was significantly increased for males at 800 ppm. Eye opening and pinna unfolding were delayed among F_2 offspring at 800 ppm. The observed changes in offspring organ weights are attributable to reductions in body weight at 800 ppm. There were no treatment-related macroscopic pathology findings in offspring from either generation.

The LOAEL for parental systemic toxicity was 800 ppm (equal to 51.0 mg/kg bw/day in males and 60.1 mg/kg bw/day in females), based on observed reductions in body weight, body weight gain and food consumption. **The NOAEL was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females).**

The LOAEL for offspring toxicity was 800 ppm (equal to 51.0 mg/kg bw/day in males and 60.1 mg/kg bw/day in females), based on significant reductions pup weights in both generations, reductions in litter size, and viability and weaning indices among F_2 offspring as well as significant delays in the age to attain vaginal opening and preputial separation. **The NOAEL was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females).**

The LOAEL for reproductive toxicity was 800 ppm (equal to 51.0 mg/kg bw/day in males and 60.1 mg/kg bw/day in females), based on observed reductions in litter weights and individual pup weights on the day of delivery (lactation day 0). **The NOAEL was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females).**

This study is acceptable and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800); OECD 416 in the rat.

Dose and Endpoint for Risk Assessment: 17.9 mg/kg/day based on significant reductions pup weights in both generations, reductions in litter size, and viability and weaning indices among F_2 offspring as well as significant delays in the age to attain vaginal opening and preputial separation the LOAEL of 51.0 mg/kg/day.

Comments about Study/Endpoint: Although a 21-day dermal study indicated that no effects were observed at dose levels up to 1000 mg/kg/day, the 21-day dermal study was not selected because of the concern for offspring toxicity demonstrated in the 2-generation reproduction study. The dermal study does not measure for the effects observed in pups in the 2-generation reproduction study. Therefore, the 2-generation reproduction study was selected for the dermal endpoints. Because offspring toxicity could occur at any time during the period of organogenesis and also because no data are available to indicate how much exposure will induce the observed effects in the reproduction study, this endpoint was selected for assessment of risk to humans for both short- and intermediate-term durations. Since an oral NOAEL was selected, a 30% dermal absorption factor should be used for route-to-route extrapolation.

2.3.4 Long-Term Dermal (Longer than 6 Months) Exposure

Study Selected: Chronic/Oncogenicity Study in the Rat § 870.4300

MRID Nos.: 44988430; 44988429, 45245304

Executive Summary: See chronic dietary section

Dose and Endpoint for Risk Assessment: 7.1 mg/kg/day based on decrease in mean body weight, body weight gain and hepatocellular vacuolation at the LOAEL of 17.5 mg/kg/day.

Comments about Study/Endpoint: This study/dose/endpoint was selected for establishing the chronic RfD. Since an oral NOAEL was selected, a 30% dermal absorption factor should be used for route-to-route extrapolation.

2.3.5 Short- (1 Day - 1 Month) and Intermediate-Term Inhalation (1 Month - 6 Month) Exposure

Study Selected: 2-Generation Reproduction Study in the Rat § 870.3800

MRID No.: 44988430

Executive Summary: See short- and intermediate-term dermal exposure section

Dose/Endpoint for Risk Assessment: 17.9 mg/kg/day based on significant reductions pup weights in both generations, reductions in litter size, and viability and weaning indices among

F_2 offspring as well as significant delays in the age to attain vaginal opening and preputial separation the LOAEL of 51.0 mg/kg/day.

Comments about Study/Endpoint: No inhalation study is available. Therefore, an extrapolation from an oral endpoint will be used. The 2-generation reproduction study was selected for the inhalation endpoints. Because offspring toxicity could occur at any time during the period of organogenesis and also because no data are available to indicate how much exposure will induce the observed effects in the reproduction study, this endpoint was selected for assessment of risk to humans for both short- and intermediate-term durations. Since an oral NOAEL was selected, a 100% inhalation absorption factor should be used for route-to-route extrapolation.

2.3.6 Long-term Inhalation (Longer than 6 Months) Exposure

Study Selected: Chronic/Oncogenicity Study in the Rat § 870.4300

MRID Nos.: 44988430; 44988429, 45245304

Executive Summary: See chronic dietary section

Dose and Endpoint for Risk Assessment: 7.1 mg/kg/day based on decrease in mean body weight, body weight gain and hepatocellular vacuolation at the LOAEL of 17.5 mg/kg/day.

Comments about Study/Endpoint: This study/dose/endpoint was selected for establishing the chronic RfD. Since an oral NOAEL was selected, a 100% inhalation absorption factor should be used for route-to-route extrapolation.

2.3.7 Margins of Exposure for Occupational/Residential Risk Assessments

Acceptable Margins of Exposure (MOEs) for occupational risk assessments are 100 for all routes and durations. The acceptable MOEs for residential exposure will be determined by the FQPA Safety Factor Committee.

2.4 Recommendation for Aggregate Exposure Risk Assessments

Common toxicological effects (reduction in pup weights, litter size, viability and weaning indices; as well as significant delays in the age to attain vaginal opening and preputial separation) were selected for assessment of short- and intermediate-term exposures by dermal and inhalation routes. The aggregate risk assessment for these exposure durations should include dermal and inhalation exposures appropriate to the populations of concern. Short-term oral exposure cannot be aggregated due to a different toxicological endpoint (decrease in body weight and body weight gain).

Common toxicological effects (reduction in mean body weight and body weight gain and hepatocellular vacuolation) were selected for assessment of long-term oral, dermal (oral equivalent) and inhalation (oral equivalent) routes. The aggregate risk assessment for this exposure duration should include oral, dermal and inhalation exposures appropriate to the populations of concern.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID Nos. 44988429, 45245304

Executive Summary: In a chronic toxicity/oncogenicity study (MRID 44988429 & 45245304), 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.

There were no treatment-related effects on mortality; eyes; hematology, clinical chemistry or urinalysis parameters; or gross findings in either sex administered any dose of the test material. Clinical signs that were observed at significantly increased incidences in treated animals included rales in high dose males (7/48 vs 0/46 for controls) during weeks 66-78 and at all doses in males during weeks 79-91 (0/44, 8/49, 19/45, and 17/48 at 0, 160, 400, and 1000 ppm, respectively). Also in high-dose male rats, the incidences of labored breathing (15/48 vs 5/46 for controls, $p<0.05$) was increased during weeks 66-78, red material around the nose during weeks 1-13 (7/60 vs 0/60 for controls) and weeks 92-104 (5/46 vs 0/37), and hunched posture (5/46 vs 0/37) during weeks 92/104. The lack of pathologic correlates indicate that the clinical signs are not biologically significant.

Treatment-related effects on body weight, body weight gain, and food consumption were observed in both sexes. High-dose male rats weighed 10-13% ($p<0.01$) less than controls throughout the study, gained 44% less weight during week 1, 14% less during the first year and 18% less over the entire study. High-dose group males also consumed 19% ($p<0.01$) less food (g/animal/day) during week 1 and 4-9% ($p<0.01$ or <0.05) less at different time points during the remaining weeks of the study. Food efficiency measured during the first 14 weeks was reduced for males in all dose groups during the first week of the study and showed an inconsistent pattern for the remaining 13 weeks. Mid-dose female rats weighed 4-17% ($p<0.01$) less than controls throughout the study and high-dose females weighed 6-27% ($p<0.01$) less. Mid- and high-dose group females, respectively, gained 27 and 42% less weight than controls during week 1, 15% and 32% less during the first year, and 16% and 23% less over the entire study. Food consumption was 6-10% and 9-19% less for mid- and high-dose group females, respectively, for most of the study. Food efficiency was reduced for mid- and high-dose group females during week 1 and showed inconsistent patterns for the remaining 13 weeks.

The postmortem examination showed statistically significant changes in absolute and/or relative weights of several organs in high-dose group male and female rats, and these changes are attributed to the decreased terminal body weight. Treatment-related microscopic changes were observed in the liver, kidney, and mammary glands. Trace to mild hepatocyte hypertrophy in the liver of mid- and high-dose male rats and high-dose group female rats at interim sacrifice and in the main study groups is considered an adaptive response rather than an adverse effect. Hepatocyte vacuolation also was observed in mid- and high-dose group male rats; the incidence was 10/12 and 10/11, respectively, compared with 2/12 for controls at interim sacrifice and 22/48 and 29/48, respectively, compared with 10/48 for controls in the main study. An increased incidence of microconcretions in the kidney papilla was noted for high-dose male rats (37/49 vs 17/48 for controls, $p<0.01$) in the main study. The incidence of 24/49 ($p<0.05$) for mammary hyperplasia in high-dose group females compared with 14/49 for controls appeared to be treatment related, but the toxicologic significance of this finding is uncertain.

The lowest-observed-adverse-effect (LOAEL) for NI-25 is 400 ppm (17.5 mg/kg/day for males and 22.6 mg/kg/day for females) for male and female rats based on reduced body weight and body weight gain for females and hepatocellular vacuolation for males. The no-observed-adverse-effect level (NOAEL) is 160 ppm (7.1 mg/kg/day for males and 8.8 mg/kg/day for females)

At the doses tested, there was some evidence of a treatment-related increase in tumor incidence when compared to controls. The incidence of mammary adenocarcinoma was significantly increased in females (9/49, 10/49, 15/47 (32%), and 17/49 (35%, $p<0.05$) for 0, 160, 400, and 1000 ppm, respectively) . The incidence of 32% at the mid dose and 35% at the high dose exceeded that of historical controls at the testing laboratory, MPI (13.3-28.6%), but was within range of historical controls for Charles River Laboratories (0-37.2%). Dosing was considered adequate based on significantly decreased mean body weight gain when compared to the control groups in both sexes and an increased incidence of hepatocyte vacuolation in male rats.

This chronic toxicity /oncogenicity study in the rat is **Acceptable/Guideline** and satisfies the guideline requirements for a chronic toxicity/oncogenicity oral study [OPPTS 870.4300 (§83-5)] in the rat. No deficiencies were noted for this study.

Discussion of Tumor Data: 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 tumor incidence was 1/47, 2/50, 0/48, and 5/48 for 0, 160, 400, and 1000 ppm, respectively. Historical control data were not provided for testes interstitial cell adenoma.

Female rats had a significant increasing trend in combined mammary gland adenomas and/or adenocarcinomas at $p < 0.05$. Although the trends for either adenomas or adenocarcinomas were close but not significant at $p < 0.05$, there were no significant pairwise comparisons for either adenomas, adenocarcinomas or combined adenomas/adenocarcinomas. The incidences of mammary

adenocarcinomas combined were 10/59, 11/60, 16/59, and 17/60. The incidence of 27% for the mid-dose and 28% for the high-dose groups exceeded the historical control, although it did not reach statistical significance. 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. Historical control data were not provided for mammary adenomas.

There were significant differences in the pair-wise comparisons of the 400 ppm dose group with the controls for pituitary adenomas, and adenomas and/or adenocarcinomas combined, both at $p < 0.05$ but not at the high dose of 1000 ppm. The incidence of pituitary adenomas and adenocarcinomas combined in females was 39/60, 41/59, 50/59, and 46/60. There were no historical control data reported for pituitary adenomas and/or adenocarcinomas in females.

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on significantly decreased mean body weight gain when compared to the control groups in both sexes and an increased incidence of hepatocyte vacuolation 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, gained 44% less weight during week 1, 14% less during the first year and 18% less over the entire study. High-dose group males also consumed 19% ($p<0.01$) less food (g/animal/day) during week 1 and 4-9% ($p<0.01$ or <0.05) less at different time points during the remaining weeks of the study. Mid-dose female rats weighed 4-17% ($p<0.01$) less than controls throughout the study and high-dose females weighed 6-27% ($p<0.01$) less. Mid- and high-dose group females, respectively, gained 27 and 42% less weight than controls during week 1, 15% and 32% less during the first year, and 16% and 23% less over the entire study. Food consumption was 6-10% and 9-19% less for mid- and high-dose group females, respectively, for most of the study. Hepatocyte vacuolation was observed in mid- and high-dose group male rats; the incidence was 10/12 and 10/11, respectively, compared with 2/12 for controls at interim sacrifice and 22/48 and 29/48, respectively, compared with 10/48 for controls in the main study. An increased incidence of microconcretions in the kidney papilla was noted for high-dose male rats (37/49 vs 17/48 for controls, $p<0.01$) in the main study.

In the subchronic feeding study in rats, at 800 ppm (50.8/56.0 mg/kg/day (M/F)) decreases in mean absolute body weights were observed in males from weeks 1-12 (90-92% of controls; $p<0.05$; 0.01 except week 11) and in females during weeks 6-13 (89-90%; statistically significant at weeks 6-8; $p<0.05$). During the treatment period, 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 in 800-ppm males at week 1 (80% of controls; $p<0.01$) and in 800 ppm females at weeks 1-7, 10, 12, and 13 (80-91% of controls; statistically significant at weeks 2 and 3: $p<0.05$; 0.01). No statistically significant differences were observed in mean food efficiencies.

In animals fed 1600 ppm (HDT: 99.9/117.1 mg/kg/day (M/F), males and females had decreases in mean absolute body weights at each week of treatment (85-87%; $p<0.05$; 0.01 for males; 77-90%; $p<0.01$ for females), with final mean absolute body weights being 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 high-dose males during weeks 1-7 (78-91% of controls; significant at weeks 1, 2, and 7; $p<0.01$), and in high-dose females during weeks 1-13 (73-91% of controls; significant at weeks 1-7 and 11; $p<0.05$; 0.01). Mean food efficiency was statistically ($p<0.05$; 0.01) decreased in high-dose males at weeks 1 and 6 (52 and 79% of controls, respectively), and in high-dose females at weeks 1, 3, and 6 (41, 66, and 47% of controls, respectively). Increased levels of total cholesterol were observed in high-dose 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/oncogenicity study in rats and the subchronic feeding study in rats, the high dose level of 1000 ppm in the chronic/oncogenicity study is considered to be adequate.

3.2 Carcinogenicity Study in Mice

MRID Nos. 44988428, 45245305

Executive Summary: In an oncogenicity study (MRID 44988428), acetamiprid (99.7% a.i., Lot #: NNI-01) was administered to groups of 50 male and 50 female Crl: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.

Survival rates were similar between the treated and control groups of both sexes. Decreased defecation was observed in 12/60 high-dose males and 11/60 high-dose females compared with none of the controls or other treated groups during weeks 1-13.

At the high dose, for the first 90 days, mean body weight gains were 57% and 43% ($p < 0.01$) of the control values for males and females, respectively. During the first 48 weeks of the study in this group, mean body weight gains were 50% and 55% ($p < 0.01$) of the controls values for males and females, respectively, but were similar to the controls (all groups remained at relatively stable weights) during the second year of the study. 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. Thus, the initial reduction in body weight gains were sufficient to cause the absolute body weights of the high-dose males and females to be significantly less than the control values throughout the study.

Body weights and body weight gains of the low-dose males and females and mid-dose females and food consumption of the mid-and low-dose groups were similar to the controls. Body weights of the mid-dose males were slightly less than that of the controls throughout the study with statistical significance ($p \leq 0.05$ or 0.01; 94-97% of controls) attained at most timepoints. Weight gain by the mid-dose males was significantly ($p \leq 0.01$; 86% of control) less than that of the control for the week 0-13 interval, but by the end of the first year (weeks 0-48), weight gain was similar to the control value.

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 and was <85% of the control levels during weeks 1-13. Food efficiencies were significantly ($p \leq 0.01$) less than the control values for high-dose males at weeks 1-4 and for high-dose females at weeks 1-3. Food efficiency for the mid-dose males was slightly (n.s.) less than that of the controls at week 1 and significantly ($p \leq 0.01$) less than that of the controls at week 2.

In males surviving to 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). 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. In the controls, amyloidosis was observed only in the jejunum of 1/37 males. The significant incidence rates of amyloidosis in various organs of the mid- and high-dose males ranged from 12.8% to 17.9% compared to 0% to 2.6% in the controls.

Therefore, the LOAEL for male mice is 400 ppm in the diet (65.6 mg/kg/day), based on decreased body weights and body weight gains and amyloidosis in numerous organs. The LOAEL for female mice is 1200 ppm in the diet (214.6 mg/kg/day) based on decreased body weights and body weight gains. The NOAEL for males and females is 130 ppm (20.3 mg/kg/day) and 400 ppm (75.9 mg/kg/day).

Treatment for up to 78 weeks with acetamiprid did not result in a significant increase in the incidence of neoplastic lesions in this 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 (MRID 45245305). Dosing was considered adequate based on decreased body weight gain and microscopic lesions in the high-dose group.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200, (§83-2a)] in mice.

Discussion of Tumor Data: 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.

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on decreased body weight gain and microscopic lesions in the high-dose group. At the high dose of 1200 ppm (186.3/214.6 mg/kg/day (M/F)), for the first 90 days, mean body weight gains were 57% and 43% ($p < 0.01$) of the control values for males and females, respectively. During the first 48 weeks of the study in this group, mean body weight gains were 50% and 55% ($p < 0.01$) of the controls values for males and females, respectively, but were similar to the controls (all groups remained at relatively stable weights) during the second year of the study. 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 and was <85% of the control levels during weeks 1-13. Food efficiencies were significantly ($p \leq 0.01$) less than the control values for high-dose males at weeks 1-4 and for high-dose females at weeks 1-3. In males surviving to 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). In the controls, amyloidosis was observed only in the jejunum of 1/37 males. The significant incidence rates of amyloidosis in various organs of the mid- and high-dose males ranged from 12.8% to 17.9% compared to 0% to 2.6% in the controls.

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 oncogenicity study in mice and the subchronic feeding study in mice, the high dose level of 1200 ppm in the oncogenicity study is considered to be adequate.

3.3 Classification of Carcinogenic Potential

The HIARC referred Acetamiprid to the Cancer Assessment Review Committee (CARC) for assessment of carcinogenic potential due to evidence of potential carcinogenicity in rats.

4 MUTAGENICITY

Acetamiprid tested negatively in a *Salmonella typhimurium* (Ames) assay, a forward mutation assay in Chinese hamster ovary cells, an *in vivo* chromosome aberration assay in Sprague-Dawley (CD) rats, a mouse micronucleus assay, and in repeat assays for unscheduled DNA synthesis (UDS) in rat liver primary cell cultures. Acetamiprid tested positively in an *in vitro* mammalian chromosome aberration assay in Chinese hamster ovary (CHO) cells.

In repeat reverse gene mutation assays in bacteria (MRID 44651849), four histidine auxtrophic (*his* $^-$) strains of *Salmonella typhimurium* (TA100, TA1535, TA98, TA 1537) and the WP2 uvrA (tryptophane auxotroph, *try* $^-$) strain of *Escherichia coli* were pre-incubated for 5 hours, then exposed to concentrations of the test substance ranging from 313 to 5000 µg/plate for 65.5 hours at 37° C, in the presence and absence of purchased metabolic activation prepared from the livers of Sprague-Dawley male rats treated with 5, 6-benzoflavone and phenobarbital plus co-factors (S9 mix). In addition to cultures treated with vehicle (DMSO), others were exposed to strain-specific and activated-specific mutagens, to serve as positive controls. N1-25 was tested up to 5000 µg/plate without any evidence of cytotoxicity or precipitation. **There was no increase in the number of revertants in either of the two main experiments.** Therefore N1-25 is considered negative for mutagenicity in these experiments.

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.

In independently performed mammalian forward cell gene mutation assays (MRID 44651857) Chinese hamster ovary (CHO) cells functionally hemizygous at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus were exposed to acetamiprid (99.9%) in dimethylsulfoxide (DMSO) at 500-4000 µg/mL -S9 or 250-3500 µg/mL +S9, with or without S9 activation derived from Aroclor 1254-induced rat livers. Forward cell mutation was monitored after exposure to selective medium permitting only mutant colonies to grow. Acetamiprid was tested up to toxic concentrations (4000 µg/mL -S9; ≥ 2750 µg/mL +S9) but at no dosage were significantly increased mutant frequencies over solvent controls observed either in the absence or presence of metabolic activation. In contrast, the positive controls induced highly significant increases in mutant frequency in both the absence and presence of S9 mix. **It is concluded that under the conditions of the assay, acetamiprid did not demonstrate mutagenic potential in this *in vitro* mammalian cell gene mutation assay.**

This study is classified as acceptable and satisfies the FIFRA Test Guideline for mammalian cell gene mutation data.

In an *in vivo* chromosome aberration assay (MRID 44651854), groups of 10 Sprague-Dawley (CD) rats (5M:5F/group) were administered a single oral dose of NI-25 (acetamiprid, 99.46%) suspended in arachis oil BP at the "maximum tolerated level of 250 mg/kg", killed 6, 24 and 48 hours later, and bone marrow prepared on glass slides and stained. Bone marrow cells were scored for the conventional array of chromosome aberrations by metaphase analysis of NI-25 (acetamiprid) following colchicine as the mitotic inhibitor. In addition to the vehicle (arachis oil) administration as negative controls harvested at 6, 24 and 48 hours, a group of 5M and 5F was administered the clastogen, cyclophosphamide (dissolved in distilled water), orally and bone marrow prepared for chromosome analysis 24 hours after dosing. NI-25 (acetamiprid) produced clinical toxicity in most of the animals at 6 and 24 hours after dose administration of 250 mg/kg (hunched posture, lethargy, decreased and labored respiration, body tremors and ptosis, plus 2 deaths, 1 each from 6-hour and 24-hour animals). In addition, a significant ($p < 0.05$) reduction in the mean mitotic index was observed at 48 hours. **NI-25 induced no significant dose-related increase in chromosome aberrations in bone marrow cells over background (vehicle control) at any of the three time points**, compared to the significantly increased positive results in cyclophosphamide-treated animals. Therefore NI-25 is considered nonclastogenic to rat bone marrow cells *in vivo* according to the study procedure. In addition, NI-25 did not induce a significant increase in the number of polyploid cells, resulting only in a high of 0.8%, which was within the background range.

Although only one NI-25 dose was assayed (the maximally tolerated level), this study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

In an *in vitro* mammalian chromosome aberration assay (MRID 44651855), cultures of Chinese hamster ovary (CHO) cells were exposed to 175, 350 and 700 µg/mL acetamiprid (NI-25, 99.2%) dissolved in dimethylsulfoxide (DMSO) for 13 or 25 hours continuously in the absence of metabolic activation, and to 337.5, 675 and 1350 µg/mL for 3 hours in the presence of metabolic activation provided by liver microsomes induced by 5, 6-benzoflavone and phenobarbital. Both numerical and structural aberrations were assayed. In addition to cultures treated with the vehicle (DMSO) as solvent (negative) control, other cultures were exposed to mitomycin C and benz(a)pyrene, to serve as positive controls for the nonactivation and activation test series, respectively. NI-25 was tested up to slight to moderate cytotoxic concentrations (700 µg/mL -S9, 1350 µg/mL +S9), namely, reduced mitotic index and reduced cell cycle progression. The investigators recorded increased structural chromosomal aberration slightly over solvent control (< 0.05) in the absence of metabolic activation, and significantly, with dose-relationship (at 675 and 1350 µg/mL). Increased structural activations consisted of chromatid breaks and exchanges under metabolic activation. Both positive controls responded with significantly increased aberration frequencies. **Hence NI-25 is a clastogen in the *in vitro* assay with the CHO test system.**

This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline for *in vitro* cytogenetic data.

In a mouse micronucleus assay (MRID 44651852) groups of 5 male and 5 female CD-1 (ICR) mice were administered N1-25 once orally at 20, 40 and 80 mg/kg suspended in 0.5% carboxymethylcellulose (CMC). Approximately 24, 48 and 72 hours after dosing, bone marrow cells were processed for the presence of micronuclei in their polychromatic erythrocytes (mPCE). The ratios of PCE to normochromatic erythrocytes (NCE) were also determined. In addition to animals dosed with vehicle (CMC) as negative controls and bone marrow harvested at 24, 48 and 72 hours, a group of 5 male and 5 female mice were dosed with the mutagen, cyclophosphamide and bone marrow cells were harvested 24 hours after dosing, to serve as positive control. The test article was assayed up to levels of clinical toxicity (death and tremors at 80 mg/kg). **There were no significant increases in micronucleated polychromatic erythrocytes (or the ratio of PCE:NCE) at any test dose or harvest period,** in contrast to significantly greater increases in mPCE in cyclophosphamide-treated bone marrow cells. Hence, N1-25 (acetamiprid) may be considered negative for clastogenicity in the mouse bone marrow micronucleus test.

This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for *in vivo* cytogenetic mutagenicity data.

In an *in vivo/in vitro* unscheduled DNA synthesis (UDS) assay (MRID 44651853) groups of three male Sprague-Dawley rats were administered single doses of acetamiprid (99.9%) suspended in 0.5% carboxymethylcellulose (CMC) by oral gavage at levels of 75, 150 and 300 mg/kg, and primary hepatocyte cultures scored for nuclear silver grain counts as a measure of UDS 2-4 and 12-16 hours after dose administration. In addition to males administered the vehicle, CMC, serving as negative controls, groups of three rats were given the mutagen, dimethylnitrosamine (DMN), also orally, to serve as positive controls. Acetamiprid was tested for UDS up to clinical toxicity, involving only one animal in the high dose (300 mg/kg) group exhibited lethargy and tremors at sacrifice, but was not used for hepatocyte harvest. Livers of perfused rats at this dose were reported to be darker than the livers from other dose groups. At higher doses (400 mg/kg) animals showed signs of lethargy, tremors and lacrimations. All the hepatocyte mean net nuclear grain counts were elevated over the CMC counts, in contrast to the marked increase in nuclear counts in hepatocytes from DMN-treated animals. Thus the investigators concluded that acetamiprid was negative for UDS in mammalian hepatocytes *in vivo*. Although there was no evidence (or a dose related positive response) that UDS, as determined by radioactive tracer procedures [nuclear silver grain counts], was induced, this study is classified as unacceptable since no toxicity was induced at the HDT (the one animal exhibiting clinical signs was not assayed for UDS), and an insufficient number of rats was used at the harvest times.

Thus this study is classified as unacceptable and does not satisfy the requirement for FIFRA Test Guideline 84-2 for genotoxic mutagenicity data. It is also not upgradable as presented.

In repeat assays for unscheduled DNA synthesis (UDS) (MRID 44651856), liver primary cell cultures from adult male Fischer 344 rats were exposed to NI-25 (99.57%) in dimethylsulfoxide at concentrations ranging from 0.500 to 5000 µg/mL (Trial 1) and 0.505 to 2020 µg/mL (Trial 2) in the presence of 10 µ Ci/mL ^{3}HTdr (42 Ci/mMole), and net nuclear labeling determined as a measure of UDS repair. Treatments above 1000 µg/mL were not analyzed for nuclear labeling due to high toxicity. Six treatments from 10 to 500 µg/mL were selected for analysis, since they covered a good range of toxicity: from 53.2% to 98.4% survival in Trial One and 64.4% to 107.5% in Trial Two. The mutagen, 2-acetylaminofluorene (AAF) was applied to additional cultures, serving as positive control and induced large increases in UDS. **None of the criteria used to indicate UDS were approached by treatment with NI-25 in either trial, and NI-25 was evaluated as inactive in the rat primary hepatocyte UDS assay, since there was no evidence (or a dose-related positive response) that UDS, as determined by radioactive tracer procedures (nuclear silver grain counts) was induced.**

This study is classified as acceptable and satisfies the requirement for FIFRA Test Guideline 84-2 for other genotoxic mutagenicity data.

5 FQPA CONSIDERATIONS

5.1 Adequacy of the Data Base

The toxicological data base is adequate for FQPA considerations. The following acceptable studies are available:

-- Acute and subchronic neurotoxicity studies
-- Developmental toxicity studies in rats & rabbits
-- Two-generation reproduction study

5.2 Neurotoxicity

In an acute neurotoxicity study (MRID # 44651842), groups of fasted, male and female Crl:CD-BR rats (10/sex/dose), were given a single oral dose of Acetamiprid (99.9%) by gavage, in 0.5% sodium carboxymethylcellulose at doses of 0, 10, 30, or 100 mg/kg bw and observed for 14 days. There were no mortalities during the study. Body weight gain and food consumption were significantly reduced in high-dose males. Body weight, body weight gain, food consumption and food efficiency were unaffected in females. Treatment with acetamiprid had no effect on brain size or weight and there was no evidence of neuropathology. Clinical signs of toxicity were limited to the high-dose animals, and included tremors, hunched posture, unsteady gait and coldness to touch. In addition, one high-dose female had slight brown nasal staining from study day 2 until termination.

High-dose males and females had significantly reduced body temperature on the day of dosing. Significantly decreased motor activity was observed in mid- and high-dose males and in high-dose

females on the day of dosing. A slight decrease in the duration of movements persisted in mid- and high-dose males on days 7 and 14. Functional observational battery evaluations revealed several treatment-related observations on the day of dosing. High-dose males exhibited tremors, difficulty in handling, walking on toes, dilated pupils and coldness to the touch. High-dose males also had decreased forelimb grip strength and hind limb foot splay. High-dose females displayed tremors, chewing, coldness to the touch and dilated pupils. High-dose females had decreased hind limb foot splay. High-dose females were seen to have abnormal gaits and/or posture, including walking on toes and hunched posture.

The LOAEL for neurotoxicity was 30mg/kg bw, based on the observed reduction in locomotor activity in males. **The NOAEL for neurotoxicity was 10mg/kg.**

This study is classified acceptable, and satisfies the guideline requirement for an acute neurotoxicity study (870.6200; OECD 424) in the rat.

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, behaviour 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).**

This study is classified acceptable, and satisfies the guideline requirement for a subchronic neurotoxicity oral study in the rat.

Evidence of neurotoxicity from other oral toxicity studies: in the subchronic feeding study in the mouse, mean absolute brain weights in females were decreased at the top two dose levels when compared to the control values. Relative mean brain weights were increased, which reflect significant decreases in body weight at these dose levels.

Developmental Toxicity

In a developmental toxicity study (MRID 44651847), acetamiprid (99.46% a.i.) was administered to 24 female Crj:CD (SD) rats/dose in 5% arabic gum and 0.01% Tween 80 in water, by gavage at dose levels of 0, 5, 16 or 50 mg/kg bw/day from days 6 through 15 of gestation. There was no mortality,

nor were there any clinical signs of toxicity noted in the study. Treatment with acetamiprid did not affect gross pathology nor cesarean section parameters. Maternal body weight, body weight gain and food consumption were reduced at 50 mg/kg bw/day, and absolute and relative liver weights were increased at 50 mg/kg bw/day. **The maternal LOAEL is 50 mg/kg bw/day, based on the observed reductions in body weight, body weight gain and food consumption and increased liver weights. The maternal NOAEL is 16 mg/kg bw/day.** Treatment with acetamiprid did not affect the number of fetuses, fetal sex ratios or fetal weights. There were no treatment related changes in fetal external nor visceral examinations. There was an increase in the incidence of the skeletal variation, shortening of the 13th rib, at 50 mg/kg bw/day. **The developmental LOAEL is 50 mg/kg bw/day, based on the increased incidence of shortening of the 13th rib. The developmental NOAEL is 16 mg/kg bw/day.**

This developmental toxicity study in the rat is classified acceptable, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat.

In a developmental toxicity study (MRID 44651848), acetamiprid (99.46% a.i.) was administered to 17 female Kbs:NZW rabbits/dose in 5% arabic gum and 0.01% Tween 80 in water, by gavage at dose levels of 0, 7.5, 15 or 30 mg/kg bw/day from days 6 through 18 of gestation. There were no treatment-related mortalities nor clinical signs of toxicity in the study. Six accidental deaths occurred among treated animals, however, these were reported to be due to dosing or handling errors. Maternal food consumption was significantly reduced at 30 mg/kg bw/day on gestation days 6-8, and a slight loss of maternal body weight was recorded among these animals over the interval of gestation days 6-10. There were no other treatment related changes observed among maternal animals. **The NOAEL for maternal toxicity is 15 mg/kg bw/day, based on decreased food consumption and body weight loss at 30 mg/kg bw/day. The maternal LOAEL is 30 mg/kg bw/day.** No signs of developmental toxicity were observed in this study. Treatment with acetamiprid did not affect the number of fetuses, fetal sex ratios or fetal weights. There were no treatment-related changes in fetal external, visceral nor skeletal examinations. **The NOAEL for developmental toxicity is 30 mg/kg bw/day, based on the lack of any treatment-related changes in any of the parameters investigated in this study.** There was no evidence of any teratogenic effects due to treatment with acetamiprid.

This developmental toxicity study in the rat is classified acceptable, and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rabbit.

5.3 Reproductive Toxicity

In a two-generation reproduction study (one litter per generation, MRID 44988430) Acetamiprid (99.9% a.i.) was administered to 26 Crl:CD BR (IGS) Sprague-Dawley rats/sex/dose in the diet at dose levels of 0, 100, 280, or 800 ppm (equal to 0, 6.5, 17.9 or 51.0 and 0, 7.6, 21.7 or 60.1 mg/kg bw/day in males and females, respectively). There were no treatment-related mortalities or clinical signs of toxicity among parental animals in either generation. In addition, there were no definitive treatment-related clinical signs among F_1 or F_2 pups. In the F_1 parental generation, two 100 ppm

females and five 800 ppm dams experienced total litter death. There was an equivocal association with the incidence of thin, pale and/or weak pups among those litters that experienced total litter death, such that the combined incidence of those clinical signs suggested a possible relationship to treatment with acetamiprid. Mean litter size (day 4 pre-cull), viability index and weaning index were significantly reduced at 800 ppm among F_2 pups. Mean litter size was also reduced among F_1 pups on lactation days 14 and 21. Body weight, body weight gain and food consumption were reduced during the premating period among males and females at 800 ppm in both generations. A slight, transient, non-adverse reduction in body weight gain and food consumption was observed in males of both generations at 280 ppm for the first few weeks (2-5) on the test diets. Maternal body weight and body weight gain were also reduced during the gestation period, however body weight gain tended to increase during the lactation period at 800 ppm. There were no treatment-related changes in reproductive function tests, including estrous cycle length and periodicity and sperm motility, count and morphology. Similarly, there were no treatment-related changes in reproductive performance in either generation. Decreases in absolute and relative organ weights at 800 ppm were attributed to the observed reduction in body weight among these animals. There were no treatment-related macroscopic or microscopic pathology findings in this study. In addition to the litter size, viability index and weaning index observations noted among offspring, significantly reduced pup weights were observed throughout the lactation period in males and females of both generations at 800 ppm. The mean age to attain vaginal opening was significantly increased for females at 800 ppm and the mean age to attain preputial separation was significantly increased for males at 280 and 800 ppm. Eye opening and pinna unfolding were delayed among F_2 offspring at 800 ppm. The observed changes in offspring organ weights are attributable to reductions in body weight at 800 ppm. There were no treatment-related macroscopic pathology findings in offspring from either generation.

The LOAEL for parental systemic toxicity was 800 ppm (equal to 51.0 mg/kg bw/day in males and 60.1 mg/kg bw/day in females), based on observed reductions in body weight, body weight gain and food consumption. **The NOAEL was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females).**

The LOAEL for offspring toxicity was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females), based on a significant delay in the age to attain preputial separation. **The NOAEL was 100 ppm (equal to 6.5 mg/kg bw/day in males and 7.6 mg/kg bw/day in females).**

The LOAEL for reproductive toxicity was 800 ppm (equal to 51.0 mg/kg bw/day in males aud 60.1 mg/kg bw/day in females), based on observed reductions in litter weights and individual pup weights on the day of delivery (lactation day 0). **The NOAEL was 280 ppm (equal to 17.9 mg/kg bw/day in males and 21.7 mg/kg bw/day in females).**

This study is acceptable and satisfies the guideline requirement for a two-generation reproductive study (OPPTS 870.3800); OECD 416 in the rat.

5.4 Additional Information from Literature Sources *(if available)*

Since this is a new chemical, it was assumed that no relevant toxicology studies would be available in the literature. A literature search was not conducted.

5.5 Determination of Susceptibility

There is no quantitative or qualitative evidence of increased susceptibility of rat or rabbit fetuses to *in utero* exposure in the developmental studies. In the rat, an increase in the incidence of shortening of the 13th rib was observed in fetuses at the same LOAEL as the dams, which exhibited reduced mean body weight, body weight gain and food consumption and increased liver weights. No developmental toxicity was observed in the rabbit at dose levels that induced effects in the does: body weight loss and decreased food consumption.

In the multi-generation reproduction study, qualitative evidence of increased susceptibility of rat pups is observed. The parental and offspring systemic NOAELs are 17.9/21.7 (M/F) mg/kg/day and the offspring/parental systemic LOAELs are 51.0/60.1 mg/kg/day based on a decrease in mean body weight, body weight gain and food consumption in the parents and significant reductions pup weights in both generations, reductions in litter size, and viability and weaning indices among F_2 offspring as well as significant delays in the age to attain vaginal opening and preputial separation in the offspring. The offspring effects are considered to be more severe than the parental effects.

5.6 Recommendation for a Developmental Neurotoxicity Study

The requirement for a developmental neurotoxicity study is recommended due to a structure-activity relationship to other known neurotoxicants and due to evidence of neurotoxicity (decreased locomotor activity) in the acute mammalian neurotoxicity study. .

5.6.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

In the acute neurotoxicity study, clinical signs of neurotoxicity were observed on the day of dosing.

Acetamiprid is structurally related to thiamethoxam and imidacloprid, both of which are neonicotinoids. Imidacloprid is a chloronicotinyl compound and is an analog to nicotine. Studies in the published literature suggest that nicotine, when administered causes developmental toxicity, including functional deficits, in animals and/or humans that are exposed *in utero*. With imidacloprid, there is evidence that administration causes clinical signs of neurotoxicity following a single oral dose in the acute study and alterations in brain weight in rats in the 2-year carcinogenicity study. With thiamethoxam, there was also evidence of clinical signs of neurotoxicity in the acute neurotoxicity study. In addition, there are indications that thiamethoxam may affect the endocrine system.

5.6.2 Evidence that **do not** support a need for a Developmental Neurotoxicity study:

No neuropathology was observed in any study.

6 HAZARD CHARACTERIZATION

With the exception of a 28-day inhalation study and a developmental neurotoxicity study, the toxicology database for acetamiprid is complete. The scientific quality is relatively high and the toxicity profile can be characterized for all effects, including potential developmental, reproductive, carcinogenic and neurotoxic effects. The acute toxicity data indicate the acetamiprid is moderately toxic via the oral route (Toxicity Category II) and is minimally toxic via the dermal and inhalation routes (Toxicity Category III). It is neither irritating to the eye nor the skin and is not a sensitizer under the conditions of the study.

Acetamiprid does not appear to have specific target organ toxicity. In all species tested, generalized nonspecific toxicity was observed as decreases in body weight, body weight gain, food consumption and food efficiency when estimated. Generalized effects were also observed in the liver in the mouse and rat studies in the form of hepatocellular hypertrophy in both species and hepatocellular vacuolation in the rat. Hepatocellular hypertrophy was observed in the rat subchronic feeding study at 50 mg/kg/day and above and in the rat chronic feeding study at 17.5 mg/kg/day and above at both the 12-month sacrifice and at study termination. In mouse studies, hepatocellular hypertrophy was observed at 430 mg/kg/day at 90 days and at 186 mg/kg/day at 12 and 18 months. These effects are considered to be adaptive effects. Hepatocellular vacuolation was observed at 17.5 mg/kg/day in the rat chronic study. In light of the lack of major liver effects in the rat studies, it is likely that this effect is more related to liver activity in response to the presence of the chemical rather than frank toxicity. Other effects observed in the oral studies include amyloidosis of multiple organs in the mouse oncogenicity study, tremors in high dose females in the mouse subchronic study, and microconcretions in the kidney papilla and mammary hyperplasia in the rat chronic feeding/oncogenicity study.

No effects were observed in the 21-day dermal study in the rabbit and no inhalation studies were conducted.

A dermal absorption study with acetamiprid was determined in male rats with exposure durations of 0.5, 1, 2, 4, 10 and 24 hours. Absorption was small and increased with duration of exposure. The quantity absorbed increased with dose but the percent absorbed increased between the low and intermediate doses and decreased between the intermediate and high doses. This is an unusual pattern. Since there are no data to demonstrate that the residues remaining on the skin do not enter the animal, then as a conservative estimate of dermal absorption, residues remaining on the skin were added to the highest dermal absorption value (6.34% at 24 hours). The residue remaining on the skin at 24 hours was 25.0% of the dose. Therefore, the potential total absorption at 24 hours was estimated to be 25.0 + 6.34 or approximately 30%. A more accurate estimate may be obtained with a repeat study with an extended exposure duration to measure absorption of the residues remaining on the skin.

The data indicated no quantitative or qualitative evidence of increased susceptibility of rat or rabbit fetuses to *in utero* exposure in the developmental studies; however, there was a qualitative increase in susceptibility of rat pups in the two-generation reproduction study.

There was no specific evidence that acetamiprid induces any endocrine disruption; however, it is noted that significant delays in preputial separation and vaginal opening were observed in pups in the two-generation reproduction study.

Acetamiprid tested negatively in a *Salmonella typhimurium* (Ames) assay, a forward mutation assay in Chinese hamster ovary cells, an *in vivo* chromosome aberration assay in Sprague-Dawley (CD) rats, a mouse micronucleus assay, and in repeat assays for unscheduled DNA synthesis (UDS) in rat liver primary cell cultures. Acetamiprid tested positively in an *in vitro* mammalian chromosome aberration assay in Chinese hamster ovary (CHO) cells.

In the chronic feeding/oncogenicity study in the rat, at the doses tested, there was some evidence of a treatment-related increase in tumor incidence when compared to controls. The incidence of mammary adenocarcinoma was significantly increased in females (9/49, 10/49, 15/47 (32%), and 17/49 (35%, $p<0.05$) for 0, 160, 400, and 1000 ppm, respectively). The incidence of 32% at the mid dose and 35% at the high dose exceeded that of historical controls at the testing laboratory, MPI (13.3-28.6%), but was within range of historical controls for Charles River Laboratories (0-37.2%). There was no indication of potential carcinogenicity in the mouse. This chemical has been referred to the Cancer Assessment Review Committee (CARC) for further review.

In an acute mammalian neurotoxicity study, a decrease in locomotor activity was observed in both sexes on the day of dosing. A slight decrease in the duration of movements persisted in some males on days 7 and 14. Functional observational battery evaluations revealed several treatment-related observations on the day of dosing. High-dose males exhibited tremors, difficulty in handling, walking on toes, dilated pupils and coldness to the touch. High-dose males also had decreased forelimb grip strength and hind limb foot splay. High-dose females displayed tremors, chewing, coldness to the touch and dilated pupils. High-dose females had decreased hind limb foot splay. High-dose females were seen to have abnormal gaits and/or posture, including walking on toes and hunched posture. No neuropathology was observed. In a subchronic mammalian neurotoxicity study, the only effects observed were related to decreases in body weight/body weight gain, food consumption and food efficiency. Again, no neuropathology was observed. Tremors in high dose female mice in the subchronic feeding study were the only other potentially neurotoxic effects observed in any other studies. This chemical is structurally related to thiamethoxam and imidicloprid, both of which are nicotinic insecticides and have similar concerns over potential neurotoxicity.

Metabolism studies indicate that absorption of orally administered acetamiprid is rapid and complete. Estimation of absorption by comparison of urinary excretion following intravenous and oral administration indicate 96-99% absorption following oral administration. This is consistent with data from the repeated dose experiments, showing 65 - 75% absorption. There do not appear to be biologically relevant gender-related differences. Pharmacokinetic parameters reflect the rapid absorption

and excretion. Tissue elimination is not greatly affected by a 50-fold dose increment. Consistent with rapid and complete excretion, the time-course in tissues is similar to that for blood. There was no evidence for sequestration of radioactivity and no significant gender-related differences. Pharmacokinetic parameters derived from the 15-day repeat dose study were similar to the single-dose study. Urinary excretion was the major route of elimination. Urinary excretion following i.v. dosing was similar to the oral route. Repeat dosing also resulted in rapid and complete urinary excretion (most within 24 hours). Fecal excretion accounted for approximately 12- 17% of a single oral or i.v. dose of the ring-labeled test article but only about 5% of the cyano-labeled material. After repeat dosing, fecal excretion accounted for between 21%-35% of the administered radioactivity. Fecal excretion of radioactivity by rats in the biliary elimination study was expectedly less; 6.72% (males) and 5.84% (females). By 48-hr, biliary elimination accounted for approximately 19% of the administered radioactivity. Under the conditions of these experiments, acetamiprid is extensively and rapidly metabolized (79-86% of the administered radioactivity). Only 3-7% of the dose was recovered in the urine and feces as unchanged test article. The initial Phase I biotransformation appeared to be demethylation of the parent compound. The most abundant metabolite identified in both sexes resulted from the removal of the cyanoacetamide group from demethylated parent. This removal (and direct removal of the group from the parent) resulted in cyanoacetamide metabolites. Urinary and fecal metabolites from the repeat dose experiment showed minor differences from the single-dose groups, the most relevant of which was a slight increase (10% of dose, both sexes vs. <4% in the single dose groups) in a particular glycine conjugate, indicating induction of metabolic enzymes with repeat exposure.

Acetamiprid 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. The European monograph states, "Neurotransmission through a nicotinic acetylocholine receptor (nAChR) is initiated from the binding of the neurotransmitter acetylcholine (Ach) to the Ach recognition site of the alpha-sub-unit, activation of its ion channel, followed by the influx of sodium ions. Acetamiprid works as antagonist of the ion channel by binding to the Ach recognition site. It is not affected by the acetylcholinesterase which degrades the natural neurotransmitter Ach. Binding affinity of acetamiprid to nAChR from vertebrate neuromuscular junctions is low (IC_{50}>300μM) whereas its binding affinity to nAChR from insects is high (IC_{50}=8.83 μM). Even though, the affinity of acetamiprid for the nAChR from the nervous system of vertebrates is also high, in insects, nAChR are present only in the central nervous system. Acetamiprid is poor in penetration through cuticle (hydrophobicity) and thus are more active on sucking hemipterian insects. When entered into the insect body, acetamiprid is not ionised and transferred easily into the central nervous system of insects where it is ionised and interacts strongly with nAChR. In contrary, penetration through the vertebrate blood brain barrier is poor, resulting in low toxicity to mammalians."

7 DATA GAPS

28-day inhalation study

Developmental neurotoxicity study

8 ACUTE TOXICITY

Acute Toxicity of Acetamiprid

GDLN	Study Type	MRID	Results	Tox Category
870.1100	Acute Oral - rat	44651833	LD_{50}: 217 mg/kg (M) LD_{50}: 146 mg/kg (F)	II
870.12	Acute Dermal - rat	44651836	LD_{50} > 2000 mg/kg	III
870.13	Acute Inhalation - rat	44651837	LC_{50}: > 1.15 mg/L (♂) > 1.15 mg/L (♀)	III
870.24	Primary Eye Irritation - rabbit	44651838	Not irritating to the eye	IV
870.25	Primary Skin Irritation - rabbit	44651839	Not irritating to the skin	IV
870	Dermal Sensitization - Guinea pig	44651840	Is not a sensitizer under conditions of study.	N/A

9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary	NOAEL= 10 UF = 100	Decrease in locomotor activity in males.	Acute mammalian neurotoxicity study
	Acute RfD = 0.10		
Chronic Dietary	NOAEL = 7.1 UF = 100	Decrease in body weight/body weight gain and hepatocellular vacuolation	Chronic feeding/ oncogenicity study in the rat
		Chronic RfD = 0.07	
Incidental Oral, Short-Term	NOAEL= 15	Decrease in body weight/body weight gain, food consumption and food efficiency. MOE = 100	13-week feeding - rat; subchronic neurotoxicity - rat; developmental rat
Incidental Oral, Intermediate-Term	NOAEL= 15	Decrease in body weight/body weight gain, food consumption and food efficiency. MOE = 100	13-week feeding - rat; subchronic neurotoxicity - rat; developmental rat
Dermal, Short-Term	oral NOAEL= 17.9	Delay in preputial separation, vaginal opening, eye opening and pinna unfolding; reduced litter size, viability and weaning indices in offspring. MOE = 100[a]	2-generation reproduction study
Dermal, Intermediate-Term	oral NOAEL= 17.9	Delay in preputial separation, vaginal opening, eye opening and pinna unfolding; reduced litter size, viability and weaning indices in offspring. MOE = 100[a]	2-generation reproduction study
Dermal, Long-Term	oral NOAEL= 7.1	Decrease in body weight/body weight gain and hepatocellular vacuolation. MOE = 100[a]	Chronic feeding/ oncogenicity study in the rat

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Inhalation, Short-Term	oral NOAEL= 17.9	Delay in preputial separation, vaginal opening, eye opening and pinna unfolding; reduced litter size, viability and weaning indices in offspring. MOE = 100[b]	2-generation reproduction study
Inhalation, Intermediate-Term	oral NOAEL= 17.9	Delay in preputial separation, vaginal opening, eye opening and pinna unfolding; reduced litter size, viability and weaning indices in offspring. MOE = 100[b]	2-generation reproduction study
Inhalation, Long-Term	oral NOAEL= 7.1	Decrease in body weight/body weight gain and hepatocellular vacuolation. MOE = 100[b]	Chronic feeding/ oncogenicity study in the rat

[a] Dermal absorption factor: 30%
[b] Assumed 100% absorption via inhalation.