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Acetamiprid

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SCIENTIFIC DATA REVIEWS
EPA SERIES 361

THE HIARC MEETING ON ACETAMIPRID WILL BE HELD ON THURSDAY, SEPTEMBER 20, 2001, ROOM 817 AT 9:00 AM

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PROPOSED DATA PRESENTATION TO HIARC

ACETAMIPRID

099050

September 20, 2001

Data Evaluation / Report Presentation

Pamela M Hurley 9/6/01
Pamela Hurley (USEPA)
Gordon Cockell (PMRA)
Toxicologists

Secondary or Peer Review

Pamela M Hurley 9/6/01
for Gordon Cockell (PMRA)
Toxicologist

PROPOSED DATA PRESENTATION TO HIARC

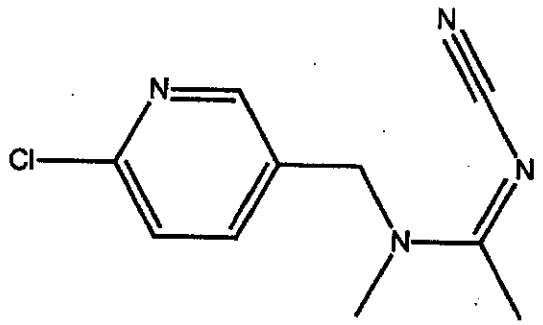
1 BACKGROUND INFORMATION

Chemical Name: Acetamiprid

Date Submitted: September 6, 2001

Acetamiprid is a new active ingredient nicotinic insecticide 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 radioligand 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 has never been to the HIARC before. The HIARC is requested to evaluate this insecticide for susceptibility under FQPA and to select toxicological endpoints for the purposes of risk assessment. The structure for acetamiprid is as follows:



The following tables summarize the use patterns and the physicochemical properties of acetamiprid. Some of these uses will be residential uses.

5

PROPOSED DATA PRESENTATION TO HIARC

Proposed Use Patterns for Acetamiprid			
Trade Name	Formulation	Method of Application	Target Crops
Adjust™	70% a.i. wettable powder	Seed Treatment	Canola seed Mustard seed
Assail™	70% a.i. wettable powder	Broadcast Foliar (aerial or ground)	Cotton Leafy Vegetables Cole Crops Fruiting Vegetables Citrus Fruits Pome Fruit Grapes
Tristar™	70% a.i. wettable powder in water-soluble pack	Broadcast Foliar (ground)	Ornamental and flowering plants grown outdoors, in greenhouses, shadehouses, and lathhouses.
Pristine™	0.006% ready-to-use liquid	Directed foliar spray	Leafy Vegetables Cole Crops Fruiting Vegetables Citrus Fruits Pome Fruit Flowers and Ornamental Plants

Physicochemical Parameters for Acetamiprid.		
Parameter	Value	Notes
Molecular Weight	222.68 g/mole	
Melting Point	98.9°C	
Density	1.33 g/cm^3	Colorless, odorless solid
Vapor Pressure	7.5 x 10^{-9} Torr at 25°C	Not volatile
Henry's Law Constant	5.17 x10^{-11} atm M^3/mole at 25°C; calculated	
Solubility at 25°C - water - acetone - ethanol - dichloromethane - hexane	4.25 g/l (4250 ppm) >200 g/l >200 g/l >200 g/l 6.54 mg/l	
Partition Coefficient (log K_{ow})	0.8	Rather hydrophylic
Soil Adsorption (Koc)	107	Range 71-138; Comparison to principal soil metabolite, IM-1-4, with K_{oc} 235, range 132-488
Water Hydrolysis - pH 5 - pH 7 - pH 9 ($T_{1/2}$)	stable stable 812 days	at 22°C; $T_{1/2}$: 420d(25°C); 53d(35°C); 13d(45°C)
Water Photolysis ($T_{1/2}$)	68 days	at 25°C, distilled and sterilized water, pH 6.5
Soil Degradation - Aerobic ($T_{1/2}$)	1 day	at 20°C, principal soil metabolite

6

PROPOSED DATA PRESENTATION TO HIARC

2 HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD - General Population including infants and children)

Proposed Study: Acute neurotoxicity study - rat Guideline #: 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.

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

Proposed Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor(s): The route and duration of exposure are appropriate for selection of the acute dietary endpoint. This study will be protective of any developmental effects observed in the rat developmental study (NOAEL/LOAEL = 16/50 mg/kg/day based on increased incidence of the shortening of the 13th rib).

7

Acute RfD =	10 mg/kg / 100	=	**0.1 mg/kg**

2.2 Chronic Reference Dose (RfD)

Proposed Studies: 2-Generation Reproduction Study — Guideline #: 870.3800

co-critical with

Chronic/Oncogenicity Study in the Rat — Guideline #: 870.4300

MRID Nos.: 44988430; 44988429, 45245304

Executive Summaries: **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

8

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 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.

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.

Proposed Dose and Endpoint for Establishing RfD: 6.5 mg/kg/day based on delay in age to attain preputial separation.

Proposed Uncertainty Factor(s): 100

Comments about Study/Endpoint/Uncertainty Factor(s): The rat chronic/oncogenicity study is selected as a co-critical study with a NOAEL of 7.1 mg/kg/day based on decreases in body weight gain in females and hepatocellular vacuolation in males. With these two studies, the general population is protected.

Chronic RfD = $\frac{6.5 \text{ mg/kg/day}}{100}$ = **0.065 mg/kg/day**

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1 - 30 days) Incidental Oral Exposure

Proposed Study: 2-Generation Reproduction Study § 870.3800

MRID No.: 44988430

Executive Summary: See chronic dietary endpoint

Proposed Dose and Endpoint: 6.5 mg/kg/day based on delay in age to attain preputial separation.

Comments about Study/Endpoint: Since 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 all durations.

2.3.2 Intermediate-Term (30 days to 6 Months) Incidental Oral Exposure

Proposed Study: 2-Generation Reproduction Study § 870.3800

MRID No.: 44988430

Executive Summary: See chronic dietary endpoint

Proposed Dose and Endpoint: 6.5 mg/kg/day based on delay in age to attain preputial separation.

Comments about Study/Endpoint: Since no data are available to indicate how much exposure will induce the effects, this endpoint was selected for assessment of risk to humans for all durations.

PROPOSED DATA PRESENTATION TO HIARC

2.3.3 Dermal Absorption

Proposed Study: Dermal Absorption Study in Rats Guideline #: 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/cm². 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%.

Proposed Percentage (%) Dermal Absorption: 30%

2.3.4 Dermal Exposure (All Durations)

Proposed Study: 2-Generation Reproduction Study Guideline #: 870.3800

MRID No.: 44988430

Executive Summary: See chronic dietary endpoint

Proposed Dose and Endpoint: 6.5 mg/kg/day based on delay in age to attain preputial separation

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 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. Since 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 all durations. An estimated dermal absorption value of 30% will be used.

2.3.5 Inhalation Exposure (All Durations)

Proposed Study: 2-Generation Reproduction Study Guideline #: 870.3800

MRID No.: 44988430

Executive Summary: See chronic dietary endpoint

Proposed Dose and Endpoint: 6.5 mg/kg/day based on delay in age to attain preputial separation

Comments about Study/Endpoint: Other than an acute inhalation study, no inhalation studies are available. Therefore, an oral endpoint was selected and a route-to-route extrapolation will be conducted. Since no data are available to indicate how much exposure will induce the effects in the reproduction study, this endpoint was selected for assessment of risk to humans for all durations. An estimated inhalation absorption value of 100% will be used.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats

MRID No.: 44988429, 45245304

Discussion of Tumor Data: 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%).

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on significantly decreased mean body weight gain in mid- and high dose females and in high dose males when compared to the control group.

3.2 Carcinogenicity Study in Mice

MRID No.: 44988428, 45245305

Discussion of Tumor Data: 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).

Adequacy of the Dose Levels Tested: Dosing was considered adequate based on decreased body weight gain in both sexes and a significant increase in the incidence of amyloidosis in numerous organs.in males.

3.3 Classification of Carcinogenic Potential

Acetamiprid is being referred to the Cancer Assessment Review Committee (CARC) for further review.

4 MUTAGENICITY

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 chromosomre 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

ACCEPTABLE STUDIES ARE AVAILABLE AS FOLLOWS:

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

THE DATA BASE IS ADEQUATE FOR FQPA PURPOSES..

5.2 Neurotoxicity Data

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.

5.3 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.

5.4 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 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.

5.5 Additional Information from Literature Sources

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.

5.6 Determination of Susceptibility

There does not appear to be any 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, quantitative evidence of increased susceptibility of rat pups is observed. The offspring systemic NOAEL is 6.5 mg/kg/day and the offspring systemic LOAEL is 17.9 mg/kg/day based on a delay in age to attain preputial separation. The parental systemic NOAEL is 17.9 mg/kg/day and the parental systemic LOAEL is 51.0 mg/kg/day based on decreased body weight, body weight gain and food consumption.

5.7 Determination of the Need for Developmental Neurotoxicity Study

5.7.1 Evidence that suggest requiring a Developmental Neurotoxicity study:

An increased quantitative susceptibility was observed in rat pups in the 2-generation reproduction study. A delay in age to attain preputial separation was observed at a dose level where no parental toxicity was observed.

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

In the subchronic feeding study in the mouse, a decrease in mean absolute brain weight was observed in females.

The active ingredient interrupts the function of the insect nervous system; however, biochemical radioligand binding studies show that acetamiprid interacts with high affinity at the insect nACHR binding site and with low affinity at the vertebrate nACHR.

5.7.2 Evidence that **do not** support the need for a Developmental Neurotoxicity study

No neuropathology was observed in any study.

6 HAZARD CHARACTERIZATION

Not applicable for proposal.

7 DATA GAPS

28-day inhalation study

21

PROPOSED DATA PRESENTATION TO HIARC

8 ACUTE TOXICITY

Acute Toxicity Profile - Acetamiprid

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

CHEMICAL: Acetamiprid
PC CODE: 099050

TOXICITY PROFILE

DER #	Guideline No./Study Type	MRID No. (year)/Classification/Doses	Results
1	870.4300 Chronic/Carcinogenicity	44988429, 45245304 1999/Acceptable 0, 7.1/8.8, 17.5/22.6, 46.4/60.0 mg/kg/day (M/F)	**NOAEL**: 7.1/8.8 mg/kg/day (M/F) **LOAEL**: 17.5/22.6 mg/kg/day (M/F, decreases in mea BW & BW gain (F) and hepatocellular vacuolation (M) Evidence of treatment-related increase in mammary tumors.
2	870.4200 Carcinogenicity - mouse	44988428, 45245305 1999, 2000/Acceptable 0, 20.3/25.2, 65.6/75.9, 186.3/214.6 (M/F) mg/kg/day	**NOAEL**: 20.3/75.9 mg/kg/day (M/F) **LOAEL**: 65.6/214.6 mg/kg/day (M/F: decreased BW & BW gain and amyloidosis in numerous organs (M) and decreased BW and BW gain (F)). Not oncogenic under conditions of study.
3	870.4100 1-Year oral - dog	44651846 1998/Acceptable 0, 9/9, 20/21, 55/61 mg/kg/day (M/F)	**NOAEL**: 20/21 mg/kg/day (M/F) **LOAEL**: 55/61 mg/kg/day (M/F: initial BW loss and overall reduction in BW gain).
4	870.3800 2-Generation Reproduction - rat	44988430 1999/Acceptable 0, 6.5/7.6, 17.9/21.7, 51.0/60.1 mg/kg/day (M/F)	**Parental systemic NOAEL**: 17.9/21.7 mg/kg/day (M/F **Parental systemic LOAEL**: 51.0/60.1 mg/kg/day (M/F (decreased body weight, body weight gain and food consumption). **Offspring systemic NOAEL**: 6.5/7.6 mg/kg/day (M/F) **Offspring systemic LOAEL**: 17.9/21.7 mg/kg/day (M/ delay in age to attain preputial separation). **Reproductive NOAEL**: 17.9/21.7 mg/kg/day (M/F) **Reproductive LOAEL**: 51.0/60.1 mg/kg/day (M/F: reductions in litter weights and individual pup weights c day of delivery).
5	870.3700 Developmental toxicity - rat	44651847 1997/Acceptable 0, 5, 16, 50 mg/kg/day	**Maternal NOAEL**: 16 mg/kg/day **Maternal LOAEL**: 50 mg/kg/day (reduced BW & BW gain and food consumption, increased liver weights). **Developmental NOAEL**: 16 mg/kg/day **Developmental LOAEL**: 50 mg/kg/day (increased incidence of shortening of the 13th rib)
6	870.3700 Developmental toxicity - rabbit	44651848 1997/Acceptable 0, 7.5, 15, 30 mg/kg/day	**Maternal NOAEL**: 15 mg/kg/day **Maternal LOAEL**: 30mg/kg/day (BW loss and decreas food consumption). **Developmental NOAEL**: 30 mg/kg/day (HDT) **Developmental LOAEL**: > 30 mg/kg/day
7	870.3100 13-Week feeding - rat	44651843 1997/Acceptable 0, 3.1/3.7, 6.0/7.2, 12.4/14.6, 50.8/56.0, 99.9/117.1 mg/kg/day (M/F)	**NOAEL**: 12.4/14.6 mg/kg/day (M/F) **LOAEL**: 50.8/56.0 mg/kg/day (M/F: decreased BW, BV gain and food consumption).
8	870.3100 13-Week feeding - mouse	44988425 1997/Acceptable 0, 53.2/64.6, 106.1/129.4, 211.1/249.1, 430.4/466.3 mg/kg/day (M/F)	**NOAEL**: 106.1/129.4 mg/kg/day (M/F) **LOAEL**: 211.1/249.1 mg/kg/day (reduced BW and BW gain, decreased glucose and cholesterol levels, reduced absolute organ weights).
9	870.3150 3-Month feeding - dog	44988424 1998/Acceptable 0, 13/14, 32/32, 58/64 mg/kg/day (M/F)	**NOAEL**: 13/14 mg/kg/day (M/F) **LOAEL**: 32 mg/kg/day (reduced BW gain in both sexes
10	870.6200 Acute neurotoxicity - rat	44651841 / 44651842 (range finding / main 1997/ Acceptable 0, 10, 30, 100 mg/kg	**NOAEL**: 10 mg/kg **LOAEL**: 30 mg/kg (reduction in locomotor activity).

TOXICITY PROFILE

DER #	Guideline No./Study Type	MRID No. (year)/Classification/Doses	Results
11	**870.6200** **Subchronic neurotoxicity - rat**	44651845 1997 / Acceptable 0, 7.4/8.5, 14.8/16.3, 59.7/67.6, 118/134 mg/kg/day (M/F)	**NOAEL:** 14.8/16.3 mg/kg/day (M/F) **LOAEL:** 59.7/67.6 mg/kg/day (M/F: reductions in BW BW gain, food consumption and food efficiency).
12	**870.3200** **21-Day dermal toxicity - rabbit**	44651844 1997/Acceptable 0, 100, 500, 1000 mg/kg/day for 6 hours/day, 5 days/week for total of 15 applications	**NOAEL:** 1000 mg/kg/day (HDT) **LOAEL:** >1000 mg/kg/day
13	**N/A** **28-Day feeding - dog**	45245306 1998/Acceptable nonguideline 0, 4.1/42.5 / 4.8/46.2, 8.4/8.7, 16.7/19.1, 28.0/35.8 mg/kg/day (M/F)	**NOAEL:** 16.7/19.1 mg/kg/day (M/F) **LOAEL:** 28.0/35.8 mg/kg/day (reduced BW gain).
14	**870.3100** **13-week feeding - rat (IM-0 Metabolite)**	44988427 1997/Acceptable 0, 9.9/11.1, 48.9/55.9, 250.1/275.9, 1246.6/1173.7 mg/kg/day (M/F)	**NOAEL:** 48.9/275.9 mg/kg/day (M/F) **LOAEL:** 250.1/1173.7 mg/kg/day (M/F: increased incidence and severity of eosinophilic intranuclear inclusions in proximal tubular epithelium of kidney (M and decreased mean BW, BW gain, food consumption and efficiency, and increased eosinophilic inclusions in kidney (F)).
15	**870.3100** **13-week feeding - rat (IM 1-4 Metabolite)**	44988426 1999/Acceptable 0, 12.8/15.6, 36.5/44.6, 112.2/135.6, 319.3/345.7-565.3 mg/kg/day (M/F)	**NOAEL:** 36.5/135.6 mg/kg/day (M/F) **LOAEL:** 112.2/345.7-565.3 mg/kg/day (M/F: increase pigment in spleen (M), decreased mean BW and BW g and increased pigment in spleen).

Acute Toxicity Profile - Acetamiprid

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

DER #1

Acetamiprid: 2-Year Feeding/Carcinogenicity Study in Rats
Nippon Soda Co. 1999. MRID No. 44988429, 45245304

DATA EVALUATION RECORD

ACETAMIPRID (NI-25)

STUDY TYPE: CHRONIC TOXICITY/ONCOGENICITY ORAL STUDY - RAT
OPPTS 870.4300 [§83-5]
MRID 44988429; 45245304

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
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Task Order No. 01-78F and .1B

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

EPA Reviewer: Esther Rinde, Ph.D., D.A.B.T. Esther Rinde, Date 8/22/01
Registration Action Branch 2 (7509C)
EPA Secondary Reviewer: SanYvette Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding- rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): NI-25 (Acetamiprid) (>99% a.i.)

SYNONYMS: none

CITATION: 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.

SPONSOR: Nippon Soda Co., Ltd., Shin-Ohtemachi Bldg., 2-1, 2-Chrome, Ohtemachi, Chiyoda-ku, Tokyo 100 Japan.

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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: NI-25

Description: pale, yellow, crystalline powder
Lot/Batch No.: NNI-01
Purity: >99 % a.i.
Stability of compound: up to 3 years
CAS No.: not provided
Structure: not provided

2. Vehicle and/or positive control

The test material was administered in the diet (Purina Certified Rodent Chow® No. 5002); no positive control was used in this study

3. Test animals

Species: rat
Strain: Crl:CD® BR
Age and weight at study initiation: 6 weeks old; males: 164 - 208 g; females: 137-157 g
Source: Charles River Laboratories, Portage, MI
Housing: The rats were housed individually in stainless-steel cages with wire mesh floors
Diet: ground Purina Certified Rodent Chow® #5002, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 21-27°C
Humidity: 40-70%
Air changes: not reported
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates

Start: October 1, 1991; end: September 28-30, and October 1, 1993

2. Animal assignment

Animals were randomly selected and assigned to the test groups in Table 1 based on homogeneity of body weights.

30

TABLE 1: Study design							
Test Group	Conc. in Diet (Ppm)	Dose to Animal (Mg/kg/day)		Main Study 24-Months		Interim Sac. 12-Months	
		Male	Female	Male	Female	Male	Female
1–Control	0	0	0	50	50	10	10
2–Low (LDT)	160	7.1	8.8	50	50	10	10
3–Mid (MDT)	400	17.5	22.6	50	50	10	10
4–High (HDT)	1000	46.4	60.0	50	50	10	10

Data taken from page 19 and 33, MRID 44988429.

3. Dose selection rationale

Doses were selected by the sponsor based on previous studies. No details were provided.

4. Diet preparation and analysis

Diets were prepared weekly by mixing an appropriate amount of test material with a small amount of feed (ground Certified Rodent Chow® #5002) to prepare a premix. After adding feed to the premix to obtain the specified concentration, the diet was mixed in a twin shell blender for 10 minutes. Control (basal diet) and test diets were stored at room temperature until used. Homogeneity and stability were tested on all dietary concentrations prepared for week 1. Ten stratified samples for each concentration were analyzed for homogeneity, and stability was determined on a composite of the ten samples stored for 10 days at room temperature. During the study, the concentration of test material in treated food was determined weekly for the first 4 weeks and at 4-week intervals thereafter.

Results –

Homogeneity Analysis: The concentrations of test material in all ten samples taken from each dietary concentration were within ±10% of the target concentration except for one 1000-ppm sample, which was 19% greater than the target concentration. The coefficients of variation for the three dietary concentrations ranged from 3.4% to 6.4%.

Stability Analysis: The concentration of test material in the composite sample of each dietary concentration after storage for 10 days at room temperature was within 3% of the concentration on day 0.

Concentration Analysis: The concentrations of test material in all samples were within -9% and +7% of the target concentrations, except for two samples that were 13% (1000 ppm) and 14% (400 ppm) greater than the target concentrations.

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

5. Statistics

Data for body weights, food consumption, food efficiency, water consumption, hematology parameters, and absolute and relative organ weights were analyzed by one-way analysis of variance (ANOVA) and Bartlett's test for homogeneity. Dunnett's multiple comparison tables were used for pairwise comparison for equal variances and the rank transformation methods for unequal variances.

Incidences of microscopic findings were analyzed using a one-tail Fisher's Exact test for interim sacrifice animals only, terminal sacrifice animals only, animals dying early and euthanized *in extremis*, and all rats combined. Statistical analysis was not conducted on findings in low- and mid-dose groups if the organ was examined because of the presence of a gross lesion. Tumor incidences were analyzed using survival adjusted and unadjusted data. The Cochran-Armitage trend and Fisher's Exact tests were used to compare the groups unadjusted for survival. Survival adjusted analysis was conducted according the prevalence/mortality method of Peto. Tumor incidence data were analyzed for all animals of each sex and dose groups (including interim sacrifice) combined.

C. METHODS

1. Observations

Animals were inspected twice daily throughout the study for signs of toxicity, morbidity, and mortality. A detailed examination with palpation for masses was conducted once a week

2. Body weight

Animals were weighed pretest, weekly for the first 14 weeks, and every 2 weeks thereafter.

3. Food consumption and compound intake

Food consumption for each animal was determined weekly for the first 14 weeks and at 2 week intervals thereafter. Mean daily diet consumption was calculated as g food/kg body weight/day and as g/animal/day. Food efficiency (body weight gain in g/food consumption in g per unit time × 100) was calculated for the first 14 weeks. Compound intake (mg/kg/day) values were calculated for the same intervals as body weight and consumption measurements.

4. Ophthalmoscopic examination

The eyes of each animal were examined pretest and at 6, 12, and 24 months of the study.

5. Blood was collected from the orbital sinus of ten randomly selected rats per group per sex at 3, 6, 12, 18, and 24 months for hematology and clinical chemistry analyses. The rats were deprived of food, but not water, overnight before blood was collected. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
	(Thromboplastin time)		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for chronic toxicity/oncogenicity based on Subdivision F Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Uric acid
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Albumin/Globulin ratio
	ENZYMES	X	Glucose*
X	Alkaline phosphatase (ALK)	X	Total bilirubin
	Cholinesterase (ChE)	X	Direct bilirubin
X	Creatine phosphokinase	X	Total serum protein*
X	Lactic acid dehydrogenase (LDH)	X	Triglycerides
X	Serum alanine aminotransferase* (SGPT)	X	Phospholipids
X	Serum aspartate amino-transferase* (SGOT)	X	Serum protein electrophoresis
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected from the same animals used for hematology and clinical chemistry and at the same time points. Urine was collected during the fasting period. The CHECKED (X) parameters were examined.

X		X	
X	Appearance*	X	Glucose*
X	Color	X	Ketones*
X	Volume*	X	Bilirubin
X	Specific gravity*	X	Blood*
X	Osmolality	X	Leukocytes
X	pH	X	Nitrite
X	Sediment (microscopic)*	X	Urobilinogen
X	Protein*		

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

7. Sacrifice and pathology

All rats included in the study (interim and main study) were subjected to a gross pathological examination. Moribund animals and those surviving to scheduled sacrifice at 12 or 24 months were euthanized by carbon dioxide asphyxiation. The study author did not indicate whether animals were fasted before sacrifice. The CHECKED (X) tissues were collected for microscopic examination. All tissues, gross lesions, and masses collected from controls, high-dose rats, all rats dying before termination, and all rats killed moribund were examined microscopically. In addition, bone with marrow, kidneys, liver, lung, pancreas, pituitary, and thyroid were examined in all low- and mid-dose group rats. Adrenal glands, eyes with optic nerve, Harderian gland, heart, sciatic nerve, lumbar spinal cord, and testes were examined in low- and mid-dose males; and mammary gland and thyroid were examined in low- and mid-dose females. The liver from males dying before 12 months or sacrificed at 12 months was examined after Periodic Acid-Schiff staining, with and without diastase. In addition, the [XX] organs from all rats killed at scheduled times were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
XX	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow* (sternum and	X	Spinal cord (3 levels)*
X	Stomach*		femur)	XX	Pituitary*
X	Duodenum*	X	Lymph nodes*	X	Eyes (optic n.)*
X	Jejunum*	XX	Spleen*		
X	Ileum*	XX	Thymus*		GLANDULAR
X	Cecum*			XX	Adrenal gland*
X	Colon*		UROGENITAL		Lacrimal gland
X	Rectum*	X	Kidneys*+	X	Harderian gland
X	Liver*+	X	Urinary bladder*	X	Mammary gland*
XX	Pancreas*	XX	Testes*+	X	Parathyroids*
X		XX	Epididymides	X	Thyroids*
	RESPIRATORY	XX	Prostate		
	Trachea*	X	Seminal vesicle		OTHER
	Lung*	XX	Ovaries*	X	Bone* (sternum and femur)
X	Nose	X	Uterus*	X	Skeletal muscle*
XX	Pharynx	X	Cervix	X	Skin*
	Larynx	X	Vagina	X	All gross lesions and masses*

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity/oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

In male rats, the appearance and frequency of clinical signs were similar in treated and control groups up to week 65. The incidence of rales was 0/46, 2/50, 4/45, and 7/48 ($p<0.01$), respectively, for surviving male rats during weeks 66-78 and 0/44, 8/49, 19/45, and 17/48 ($p<0.01$ all dose groups), respectively, during weeks 79-91. The incidence of rales was similar to that of controls from weeks 92-104. The incidence of labored breathing was increased in high-dose male rats (15/48 vs 5/46 for controls, $p<0.05$) during weeks 66-78; no significant increase in the incidence was observed at any other time during the study. According to the study author, except for two high-dose rats, labored breathing and rales occurred in different animals of the same group. A larger number of high-dose male rats had red material around the nose during the first 13 weeks (7/60 vs 0/60 controls) and during the last 13 weeks of the study (5/46 vs 0/37 controls) compared with the number in the control group. The incidence of hunched posture also was significantly ($p<0.05$) increased in high-dose males during the interval from weeks 92-104 (5/46 vs 0/37 for controls).

The incidences of rales and labored breathing in females administered the test material were similar to those of the controls. The incidence of hunched posture in high-dose females was significantly ($p<0.05$) increased from weeks 79-91 (10/46 vs 2/42 for controls).

2. Mortality

No treatment-related effect was observed on mortality in male or female rats fed any dose of the test material. Mortality was 0-5% for all groups at 12 months and 4-12% for all male groups and 6-16% for all female groups at 18 months. At study termination, mortality was 38, 20, 32, and 20% for males and 54, 48, 42, and 42% for females fed the 0, 160-, 400-, and 1000-ppm diets, respectively.

B. BODY WEIGHT

Selected data for body weights and body weight gain are presented in Table 2. With the exception of females in the second year of the study, toxicologically significant lower mean body weight gains were consistently observed in high dose rats of both sexes and in mid-dose females when compared to the control group throughout the study. Mean body weights for mid-dose females were statistically significantly less than the control group throughout the study; however, the mean value became less than 90% of the control value only in the second year of the study. No other biologically significant changes were noted.

For males, mean body weight gains for the low, mid- and high dose groups expressed as a percentage of the control value were 98, 96 and 82% at 13 weeks; 100, 102 and 86% at 52 weeks and 100, 101 and 82% at 104 weeks, respectively. For females, mean body weight gains for the low, mid- and high dose groups expressed as a percentage of the control value were 93, 84 and 71% at 13 weeks, 94, 85 and 68% at 52 weeks and 107, 84 and 77% at 104 weeks, respectively.

TABLE 2. Selected mean body weights and body weight gain data for male and female rats fed NI-25 for up to 24-months

Study Week	Dietary Concentration (ppm)							
	0	160	400	1000	0	160	400	1000
	Males				Females			
Mean body weight (g)								
0	185	185	185	185	146	146	146	146
1	242	236	230** (95)[a]	217** (90)	172	169	165** (96)	161** (94)
8	422	419	417	377** (89)	256	249	242** (95)	225** (88)
13	477	470	468	423** (89)	281	272	260** (93)	242** (86)
26	551	540	541	484** (88)	323	306	295** (91)	271** (84)
52	626	638	636	566** (90)	402	387	363** (90)	319** (79)
78	690	699	684	611** (89)	469	426	400** (85)	343** (73)
104	667	668	673	578** (87)	433	453	388 (90)	367* (85)
Body Weight gain (g)[b]								
0-1	57	51	45	32 (56)	26	23	19 (73)	15 (58)
0-13	292	285	283	238 (82)	135	126	114 (84)	96 (71)
0-26	366	355	356	299 (82)	177	160	149 (84)	125 (71)
0-52	441	453	451	381 (86)	256	241	217 (85)	173 (68)
52-104	41	60	37	12 (29)	31	66	25 (81)	48 (155)
1-104	482	483	488	393 (82)	287	307	242 (84)	221 (77)

Data taken from Table 5, pages 91-94, MRID 44988429.
[a]Numbers in parentheses are percent of control values, calculated by the reviewer.
[b]Body weight gain calculated by the reviewer.
**p<0.05, **p<0.01, statistically significant, treated groups compared with controls.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Selected food consumption data are summarized in Table 3. In male rats, food consumption (expressed as g food/kg body weight/day) by the high-dose group was significantly reduced by 9% ($p<0.01$) compared with that of controls during the first week of dosing and significantly exceeded that of controls by 3-10% ($p<0.01$ or <0.05) up to week 48. Food consumption by high-dose male rats also exceeded that of controls by 5-13% ($p<0.01$ or <0.05) during the second year; statistical significance was reached only at sporadic time points. Food consumption, expressed as g/animal/day was reduced by 19% ($p<0.01$) in high-dose group males compared with that of controls during the first week of dosing and was reduced by 4-9% at different time points throughout the study. Male rats fed the 160- and 400-ppm diets consumed similar amounts of food as the controls.

Food consumption (g food/kg body weight/day) by females in the high-dose groups was slightly but significantly reduced by 3-12% ($p<0.01$ or <0.05) during weeks 1 to 4 and varied only slightly from that of controls up to week 22. Starting at week 24, food consumption by high-dose group females significantly exceeded that of controls

37

for almost all time points until study termination. High-dose group females consumed up to 22% more food than controls. In general, mid-dose group females consumed amounts similar to that of controls except for small statistically significant decreases of 3-5% ($p<0.01$) during weeks 1 to 3 and sporadic statistically significant increases of 6-13% during the second year of the study. Based on the amount of food consumed per animal per day, mid- and high-dose group females consumed significantly ($p<0.01$ or <0.05) less food for most time points up to week 78; mid-dose females consumed 6-10% less food than controls and high-dose females consumed 9-19% less. In addition, low-dose group females consumed 3-10% ($p<0.01$ of <0.05) less food than controls at sporadic time points.

TABLE 3. Selected mean food consumption and food efficiency data for male and female rats fed NI-25 for up to 24-months

Study Week	Dietary Concentration (ppm)							
	0	160	400	1000	0	160	400	1000
	Males				Females			
Food consumption[a]								
1	97.3 23.5	98.0 23.1	95.3 21.9** (93)	88.3** (91)[b] 19.1** (81)	102.2 17.5	100.9 17.0* (97)	97.3** (95) 16.0** (91)	89.6** (88) 14.4** (82)
8	58.6 24.6	58.9 24.7	59.1 24.6	61.4** (105) 23.2** (94)	72.6 18.5	70.0 17.4* (94)	74.8 18.1	71.5 16.1** (87)
13	50.8 24.1	50.3 23.7	50.4 23.6	54.9** (108) 23.1* (96)	67.3 18.8	64.9* 17.6** (94)	66.6 17.3** (92)	67.5 16.4** (87)
26	43.2 23.8	41.6 22.4	42.9 23.2	46.9** (109) 22.7	58.9 18.9	57.6 17.6	59.0 17.4** (92)	63.4** (108) 17.1** (90)
52	34.9 21.8	35.3 22.3	34.6 21.9	36.8 (105) 20.6	44.5 17.7	45.5 17.4	47.4 17.1	50.9** (114) 16.2** (92)
78	35.1 23.9	33.9 23.5	33.1 22.2	36.2 22.0* (92)	41.1 19.0	43.8 18.6	44.2 17.7	49.5** (120) 16.9* (89)
104	32.2 21.4	33.1 21.8	32.2 21.7	34.8 19.9	39.3 16.8	41.3 18.0	44.7 17.3	47.3** (120) 17.2
1-104	43.9[c]	44.3	43.9	46.4 (106)	55.3	55.1	56.5	60.0 (108)
Food efficiency ((g body weight gain/g food consumed) × 100)								
1	30.41	27.67** (91)	25.83** (85)	20.75** (68)	18.01	16.28	14.43** (80)	12.65** (70)
8	11.89	10.94	11.33	9.72** (82)	5.14	4.45	6.75	6.53 (127)
14	5.50	5.43	5.76	4.06 (74)	4.42	3.96	4.24	3.72 (84)

Data taken from page 33 and Tables 6 and 8 (pages 95-102 and 107-108), MRID 44988429.
[a]Top row is food consumption expressed as g food/kg body weight/day and bottom row is g food/animal/day.
[b]Numbers in parentheses are percent of control values, calculated by the reviewer.
[c]Average food consumption over the entire study expressed as g food/kg body weight/day
**$p<0.05$, **$p<0.01$, statistically significant, treated groups compared with controls.

38

2. Compound consumption

The average compound consumption for each dose group is presented in Table 1.

3. Food efficiency

Food efficiency for male rats showed a dose related decrease of 9, 15, and 32%, respectively, for the low-, mid-, and high-dose groups for week 1 of the study. Thereafter, food efficiency values for male rats showed no specific trends and fluctuated from greater than the control values to less than control values for all treatment groups. Females administered the mid- and high-dose had food efficiency values of 20% and 30% less, respectively, than that of controls for week 1. Fluctuations were also observed for females after week 1. The values for high-dose females were generally less than that of controls, but no consistent dose-related trends were observed.

D. WATER CONSUMPTION

Water consumption measured during weeks 24, 50, 76, and 102 and calculated as g/animal/day was similar in treated and control rats of both sexes throughout the study. When water consumption was calculated as g/kg body weight/day, a slight increase was observed for high-dose rats of both sexes at the four time points; statistical significance was reached during week 24 for males (118% of controls, $p<0.01$) and week 78 for females (134%, $p<0.01$).

E. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects were observed on the eyes of male or female rats administered any dose of the test material.

F. BLOOD WORK

1. Hematology

The mean corpuscular hemoglobin concentration (MCHC) was increased by 6% ($p<0.05$) in the high-dose group males at study termination. This isolated small increase is not considered treatment related. Administration of the test material caused no effect on hematologic parameters in either sex at any time during the study.

2. Clinical chemistry

No notable changes were observed for clinical chemistry parameters in male or female rats receiving the test material compared with those of controls at any time during the study except as noted for high-dose female rats below. Blood urea nitrogen (BUN) levels were elevated by 25% at 3 and 6 months and decreased 25% by study termination (Table 11, Study number 449-015) . Triglycerides were decreased by 63% ($p<0.01$ or <0.05) at 12 and 18 months and by 41% (N.S.) at 24 months. The

serum globulin level was increased slightly (11%, $p<0.05$) at 18 months and 24 months (12%, not statistically signficant) and the corresponding albumin/globulin ratio was decreased by 27% ($p<0.05$) at 18 months and 10% (not statistically significant) at 24 months (Table 11, Study number 449-015).

G. URINALYSIS

No notable changes were observed for urinalysis parameters in male or female rats receiving the test material compared with those of controls at any time during the study except for a 36%, 33% and 24% increase in osmolality in high-dose group males at 6, 12 and 24 months, respectively. Only the 6 and 12 month values were statistically significantly increased over the control group.

H. SACRIFICE AND PATHOLOGY

1. Organ weight

At the 12 month sacrifice, absolute and relative (to brain) kidney weights of high-dose group male rats were 13% ($p<0.05$) and 15% ($p<0.01$) less, respectively, and absolute and relative (to brain) weight of the lungs and mainstream bronchi were 11% (N.S.) and 13% ($p<0.05$) less, respectively, than that of the controls. In the high-dose group females sacrificed at 12 months, relative (to body weight) weights of the brain (+23%), heart (+17%), kidney (+20%), liver (+18%), lung and mainstream bronchi (+21%), and spleen (+29%) were significantly ($p<0.01$ or <0.05) increased compared with the control values. The absolute weights were similar to the control organ weights. High-dose group females sacrificed at 12 months had a mean terminal body weight 19% ($p<0.01$) less than that of controls. Therefore, the increases in the relative (to body weight) weights of these organs were probably related to the drop in bodyweight.

At study termination, the relative (to body) weights of the brain (+13%), liver (+9%), and lung and mainstream bronchi (+17%) were significantly ($p<0.01$ or <0.05) increased compared with the control values. The mean terminal body weight for high-dose male rats was 13% ($p<0.01$) less than that of controls and the mean terminal body weight for high-dose females was 15% less than the control group. Therefore, these relative (to body) weight increases were probably due to the decrease in mean body weight. Absolute weight of the prostate was decreased by 26% ($p<0.01$) and the relative (to brain) weight was increased by 25% ($p<0.01$). In addition, the absolute weights of the heart (-8%) and kidney (-16%), as well as the relative (to brain) weight of the kidney (-15%) were significantly ($p<0.01$) decreased compared with the control values. The absolute weight of the heart (-10%) and thymus (-37%) and the relative (to brain) weight of the thymus (-35%) were significantly ($p<0.05$) lower than that of controls. Organ weights of low- and mid-dose group rats of either sex were not affected by treatment with the test material.

2. Gross pathology

40

No treatment-related gross findings were observed in male or female rats administered the test material. Common findings included enlarged pituitary gland in both sexes and subcutaneous masses in females.

3. Microscopic pathology

a. Non-neoplastic

Notable nonneoplastic lesions are summarized in Table 5. Trace hepatocellular hypertrophy was observed in 42% ($p<0.05$) of mid-dose male rats and trace to mild hypertrophy was observed in 91% ($p<0.01$) of high-dose male rats in the 12-month sacrifice groups (includes rats dying before 12 months and those sacrificed at 12 months). None of the 12 control or the 10 low-dose male rats examined had this lesion. Trace hepatocellular hypertrophy was observed in 36% of ($p<0.05$) high-dose female rats, but in none of the female rats in the control or lower dose groups. Trace to mild hepatocellular vacuolation was observed 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. Hepatocellular vacuolation was observed in only one control and one high-dose female rat at the 12-month interim sacrifice. The incidence of mammary galactocele (cyst containing milk) was significantly increased in mid-dose females, but not in high-dose females. The incidence of mammary galactocele was 54% ($p<0.01$) and the severity was graded mild to moderate at the mid-dose level compared with only 36% (N.S.) graded trace to mild in high-dose females. Trace mammary galactocele lesion was observed in only one female control.

In main study male rats (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 groups. The incidences in mid- and high-dose group males were 31% and 71%, respectively, for hypertrophy and 46% and 60%, respectively, for vacuolation. Hepatocyte hypertrophy was not observed in control males but hepatocyte vacuolation was observed in 21%. In addition, microconcretions in the renal papilla was observed in 76% ($p<0.01$) of high-dose males compared with 35% of controls. Microconcretions were trace to severe in the high-dose group, trace to moderate in the mid-dose group, and trace to mild in the low-dose group and the controls showing an obvious dose-related increase in severity.

In the main study female rats, trace hepatocyte hypertrophy was observed in only 3/49 (6%, N.S.) high-dose group rats, compared with none of the controls or lower dose groups. In contrast to the interim sacrifice group, neither the incidence (70% vs 57% for controls, N.S.) nor severity of mammary galactocele was significantly increased in mid-dose group females. However, incidence of mammary hyperplasia (not otherwise specified) was increased (49%, $p<0.05$) in high-dose group females in the main study 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 of the controls. The incidence of mammary hyperplasia in historical controls in female rat studies at the testing laboratory ranged from 5-59% for seven studies. Alveolar macrophages were found in the lungs of 31% ($p<0.05$) of high-dose females compared with only 12% of the controls.

TABLE 5. 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)*	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)

Data taken from Table 15, pages 177-252, MRID 44988429.
* 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.

b. Neoplastic

Notable neoplastic lesions are summarized in Table 6. None of the neoplasms in treated male rats occurred with incidences significantly higher than those of controls rats. The incidence of interstitial cell adenomas in the testis was increased in high-dose males compared with the control incidence; the pairwise statistical test did not show statistical significance, but the trend test showed marginal significance ($p=0.054$, Cochran Armitage trend test). The incidence of thyroid c-cell adenomas was increased in high-dose males, but the incidence did

42

not achieve statistical significance compared with the control incidence. The incidence at the high-dose level was just outside the range of historical controls if an outlier incidence was excluded. Pituitary adenomas occurred at a high incidence in all groups including controls; the incidences ranged from 39-53%.

In female rats, the incidence of mammary adenocarcinoma was increased in mid- and high-dose females. The incidence at the high-dose level reached statistical significance but not at the mid-dose level. The incidence of thyroid c-cell adenomas was also increased in females administered the high-dose. The incidence was slightly above that of the upper range for historical but it did not achieve statistical significance compared with concurrent controls. The incidence of pituitary adenomas was very high in all groups of female rats, ranging from 76% to 96%.

TABLE 6. Notable neoplasms in male and female rats (main study group only) fed NI-25 for up to 24 months

Organ/lesion	Dietary Concentration (ppm)			
	0	160	400	1000
Males				
No. animals examined	48 10 at interim	50 10 at interim	48 10 at interim	49 10 at interim
Pituitary Gland/Adenoma	29	32	28	22 + 1 at interim
Thyroid Gland/ C-cell adenoma C-cell carcinoma Total[a]	4 (8%) 1 at interim 2 (4%) 6 (13%) 1 at interim	5 1 6	6 1 7	9 (18%) 0 9 (18)
Historical Control Data[c]	MPI (testing laboratory): range: 0-30.44%; average: 9.33% MPI: range 0-16.13%; average 7.41% (outlier 30.44% excluded) Charles River CD®: range 2.0-16.4%			
Testes/ Interstitial cell adenoma	1 (2%)	2	0	5 (10%)
Females				
No. animals examined	49 10 at interim	49 10 at interim	47 10 at interim	49 10 at interim
Mammary Gland/Adenocarcinoma	9 (18%) + 1 at interim	10 (20%) + 1 at interim	15 (32%) + 1 at interim	17* (35%)
Historical Control Data[d]	MPI: range: 13.3-28.6%; average 20.1% CD® (Charles River): range 0-37.2%			
Pituitary Gland/Adenoma	37[b] + 1 at interim	41	45 + 3 at interim	43 + 1 at interim
Thyroid Gland/ C-cell adenoma C-cell carcinoma Total[a]	5 0 5 (10%)	5 0 5	4 2 6	8 1 9 (19%)
Historical Control Data	CD® (Charles River): range: 1.0-17.1%			

Data taken from Table 16 (pp. 323-346), MRID 44988429 and pp. 7-12, 46, and 54, MRID 45245304.
[a]The total numbers with a Thyroid gland C-cell adenoma and/or carcinoma.
[b]The pituitary was examined in all 50 animals.
[c]Studies completed after October 1993 are excluded from calculating the incidence in historical controls.
[d]All studies except one were completed after the in-life date for the current study.
*$p \leq 0.05$, statistically significant, control group compared with the control, calculated by the reviewer using Fisher's exact test.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The investigators concluded that NI-25 was not carcinogenic. The no-observed-effect level (NOEL) was 160 ppm based on increased incidences of clinical signs at 400 and 1000 ppm, decreased body weights in females at 400 and 1000 ppm and in males at 1000

were not associated with any other pathologic condition and are not considered treatment related or biologically significant. No treatment-related findings resulted from the examination of the eyes.

Postmortem examination showed no treatment-related gross lesions in either sex receiving any dose of the test material. Common gross findings included enlarged pituitary glands in both sexes associated with a high incidence of pituitary adenomas in all dose groups including controls and subcutaneous masses in females associated with mammary tumors. The absolute and/or relative weights of several organs in high-dose group male and female rats were significantly different from those of controls. The organ weight changes were due to decreased terminal body weights of high-dose male and female rats. There were no microscopic changes specifically associated with organ weight changes.

Microscopic changes in the rats administered the test material included hepatocyte hypertrophy and vacuolation in mid- and high-dose males and hepatocyte hypertrophy in high-dose female rats at 12 months and hepatocyte hypertrophy and vacuolation in mid- and high-dose males in the main study. Trace to mild hypertrophy is not considered biologically significant. The hypertrophy observed in this study is considered to be an adaptive response and the severity did not exceed mild in any animal. Hepatocyte vacuolation is considered to be an adverse lesion. The incidence at 46% in mid-dose male rats also exceeded that of historical controls (0-31%) for MPI. The incidence and severity of kidney microconcretions (also called calculi) were increased in high-dose male rats; the incidence in the treated group (76%) greatly exceeded that of historical controls (0-15%). It should also be noted that the incidence in concurrent controls (35%) exceeded that of the historical controls.

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 also author 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% for 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.

High-dose females also had a significantly increased incidence of lungs with alveolar macrophages. The incidence of this finding was not significantly increased in males or females at the 12-month interim sacrifice. Alveolar macrophages in the lungs may have been caused by inhaling very small particles of the ground treated feed into the lungs. The reviewer does not consider this finding to be treatment related.

45

p> ppm, increased food consumption at 1000 ppm, hepatocellular hypertrophy and vacuolation in males at 400 and 1000 ppm, and hepatocellular hypertrophy in females at 1000 ppm. No treatment-related effects were observed on hematologic or clinical chemistry parameters, eyes, organ weights, or gross findings.

B. REVIEWER'S DISCUSSION/CONCLUSIONS

Administration of NI-25 in the diet at concentrations up to 1000 ppm for 24 months caused no treatment-related mortality. Clinical signs associated with administration of the test material included rales, labored breathing, hunched posture, and red material around the nose in males and hunched posture in females. Most of these observations occurred during the second year of the study and particularly during the latter part of the study. Rales were observed in all male treated groups during weeks 66-78 and the incidence was statistically significant for all dose groups compared with the controls. The investigators concluded that rales at 160 ppm were not treatment related. The reviewer concludes that rales are likely related to treatment with the test material; however, the transient nature of the observation, the lack of a pathologic correlate to explain the occurrence of rales, and the lack of a statistical effect in female rats suggest that rales are not toxicologically significant in male rats at any dose. The underlying cause of rales is unknown, but they did not occur in the same rats that showed evidence of labored breathing. The study author noted that hunched posture and red/brown material around the nose occurred in the same animals that had labored breathing. Hunched posture was the only clinical sign observed in significantly greater number of high-dose female rats than in the controls. No pathologic conditions were observed in the respiratory tract to explain the occurrence of labored breathing. It is possible that the respiratory signs were due to inhalation of fine particles of the ground treated food The remaining clinical signs occurred with similar incidences in treated and control rats.

Treatment-related effects were observed in body weight gain and food consumption in male and female rats throughout the study. Body weights were generally less than the control group in high-dose males and females (first half of the study only). Body weight gains were also lower except for the latter part of the study for high-dose females that had greater weight gain than controls, which lost weight. Body weight gains for mid-dose females were also lower than the control group. Food consumption expressed as g food/kg body weight exceeded that of controls for high-dose male and female rats except for a decrease during the early part of the study. However, when food consumption was expressed a g/animal/day, the values were decreased throughout most of the study in high-dose rats of both sexes. At the high-dose level, food efficiency showed a dose related decrease for the first week in both sexes, but no specific pattern was noted for the remainder of the first 14 weeks.

Male and female rats administered the test material showed no treatment-related effects on hematology, clinical chemistry, or urinalysis. The small statistically significant increase in MCHC in 1000-ppm males at study termination is not considered treatment related because no treatment-related erythrocytes changes were observed in this study. The changes in clinical chemistry parameters, BUN, triglycerides, serum globulin level, and albumin/globulin ratio in high-dose females were small in magnitude, transient, and

In conclusion, the lowest-observed-adverse-effect level (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 in females and hepatocellular vacuolation in 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).

This study showed some evidence of carcinogenicity in female rats. In male rats the incidence of interstitial cell adenoma of the testes was increased at the high dose, but statistical significance was not achieved. Historical control data were not provided for interstitial cell adenoma. The incidence of thyroid gland c-cell adenoma was increased in the high-dose group males. Statistical significance was not achieved, and the incidence exceeds that of historical controls only if the outlier was excluded. In females, the incidence of mammary gland adenocarcinoma was significantly increased at the high-dose compared with the control incidence and exceeded that of historical controls from the testing laboratory (MPI), but was within range of historical controls for Charles River Laboratories. This analysis was based on the incidence in main study animals only (interim sacrifice animals were excluded) using Fisher's exact test for pairwise comparison with concurrent controls. The reviewer concludes that there is some evidence of carcinogenicity in female rats based on the increased incidence of mammary gland adenocarcinoma. The study author concluded that the increased incidence of mammary adenocarcinoma was not related to treatment with the test material. The study author based the incidence on all 60 females assigned to the high-dose group including those sacrificed at 12 months. The reviewer based the incidence only on those animals assigned to the main study and did not include those terminated at 12 months. Incidences in interim sacrifice animals were included on a separate line.

These animals were adequately dosed based on lower body weights and/or body weight gains at the two highest doses for female rats and the highest dose for male rats. In addition, microscopic lesions were observed in the liver of male rats at the two highest doses.

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.

C. STUDY DEFICIENCIES

No deficiencies were noted for this study.

DER #2

Acetamiprid: 18-Month Carcinogenicity Study in Mice
Nippon Soda Company. 1999. MRID No. 44988428, 45245305

DATA EVALUATION RECORD

ACETAMIPRID
(NI-25)

STUDY TYPE: ONCOGENICITY FEEDING – MOUSE [870.4200 (§83-2a)]
MRIDs 44988428, 45245305

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Task Order No. 01-78E

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Date: APR 19 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

ACETAMIPRID **Oncogenicity Study [OPPTS 870.4200 (§83-2a)]**

EPA Reviewer: Esther Rinde, Ph.D. _____, Date _____.
Science Information Management Branch
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2a)]

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): Acetamiprid (purity, 99.7% a.i.)

SYNONYMS: NI-25

CITATION: 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.

SPONSOR: Nippon Soda Company, Ltd., Shin-Ohtemachi Building, 2-1, 2-Chome, Ohtemachi, Chiyoda-ku, Tokyo 100, Japan

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.

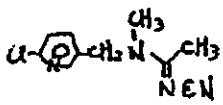
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Acetamiprid technical

   Description: white powder
   Lot #: NNI-01
   Purity: 99.7% a.i.
   Stability of compound: stable on reanalysis after study termination
   CAS #: not given
   Structure:



2. Vehicle and/or positive control: The test material was mixed with feed; a positive control was not included in this study.

3. Test animals: Species: mouse

   Strain: Crl:CD-1® (ICR) BR
   Age and weight at study initiation: 6 weeks; males: 23-28 g; females: 19-26 g
   Source: Charles River Laboratories, Portage, MI
   Housing: Animals were housed individually in stainless steel cages with wire-mesh floors.
   Diet: Certified Rodent Chow #5002 (Purina Mills, Inc., St. Louis, MO) was available

ad libitum.
Water: Water was available *ad libitum* through an automatic watering system.
Environmental conditions:
Temperature: 19-24°C
Humidity: 30-70%
Air changes: not stated
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 14 days

B. STUDY DESIGN

1. In life dates

Start: October 4, 1991; end: April 8, 1993

2. Animal assignment

Animals were assigned to the test groups in Table 1 by utilizing a block randomization procedure in which the mice were stratified by body weight. Groups were accepted if body weight variance was homogeneous. Ten animals per group were

sacrificed after 12 months and the remainder were sacrificed after 18 months of treatment.

TABLE 1. Study design

Test group	Dietary concentration (ppm)	Dose to animals (mg/kg/day)		Main study (18 months)		Interim sacrifice (12 months)	
		Male	Female	#Males	#Females	#Males	#Females
1 Control	0	0	0	50	50	10	10
2 Low	130	20.3	25.2	50	50	10	10
3 Mid	400	65.6	75.9	50	50	10	10
4 High	1200	186.3	214.6	50	50	10	10

Data taken from pp. 17 and 27, MRID 44988428.

3. Dose selection

The dose selections were based on the results of previous studies. Further details were not included in the current report.

4. Diet preparation and analysis

Test diets were prepared weekly. A premix for each dietary concentration was prepared by mixing an appropriate amount of the test article with a small amount of the diet in a Hobart mixer for 5 minutes. The premix was then blended with an appropriate additional amount of diet in a twin shell blender for 10 minutes with an intensifier bar operated for the entire blending period. Control and test diets were stored at room temperature. Homogeneity and stability analyses were conducted on all diets prepared for the first week of the study. Homogeneity of the dietary mixtures was determined from 10 stratified samples. Stability was analyzed after storage for 10 days at room temperature. Samples for concentration analyses were taken from diets prepared for weeks 1-4 and every 4 weeks thereafter.

Results –

Homogeneity: Samples from the low-, mid-, and high-concentration diets ranged from 90-102%, 93-101%, and 96-105%, respectively, of nominal.

Stability: Concentrations in the test diets after storage for 10 days at room temperature were 98-99% of their initial measured concentrations.

Concentration analysis: Throughout the study, dietary concentrations ranged from 89% to 114% of nominal with one exception. The 400-ppm diet for week 4 was found to contain 129% of nominal; subsequent analysis at week 6 showed 99% of nominal. Overall concentrations for the 130-, 400-, and 1200-ppm diets were 100%, 102%, and 99%, respectively, of nominal.

Results of the dietary analyses indicate that the test article was adequately mixed in the diets and that the actual doses to the animals were acceptable.

5. Statistics

Body weight, food consumption, food efficiency, hematology, and organ weight data were analyzed with a one-way analysis of variance and Bartlett's test for homogeneity of variance. Dunnett's multiple comparison tables or pairwise comparisons with a Bonferroni correction were used to determine significant differences. Where the non-parametric statistical procedures were appropriate, rank transformation methods were used.

Non-neoplastic microscopic findings were analyzed using Fisher's Exact test. Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated and Fisher's Exact test was used to compare each treatment group with the control. The survival adjusted test was conducted according to prevalence/mortality methods of Peto.

C. METHODS

1. Observations

Animals were observed for morbidity, mortality, and clinical signs of toxicity 3 times a day on weekdays and twice a day on weekends. Detailed clinical examinations, including palpatations, were conducted at least weekly.

2. Body weight

Animals were weighed at study initiation and weekly for the first 16 weeks of treatment followed by once every four weeks thereafter.

3. Food consumption and compound intake

Individual food consumption was measured weekly for the first 16 weeks then every 4 weeks thereafter. From food consumption and body weight values, compound consumption was calculated for the same intervals and food efficiency was calculated for weeks 1-16. Food efficiency was calculated using the equation (change in body weight [g]/food consumed [g]) × 100.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not done.

5. Blood was collected from the orbital sinus after 12 and 18 months of treatment. Clinical laboratory studies were conducted on 10 mice/sex/group. Blood smears were made from all surviving animals and differential counts were determined. The mice were not anesthetized or fasted prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count*
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for oncogenicity studies unless effects are observed, based on OPPTS 870.4200 Guidelines.

b. Clinical chemistry

Clinical chemistry tests were not conducted and are not required for oncogenicity studies based on OPPTS 870.4200 Guidelines.

6. Urinalysis

Urinalysis was not conducted and is not required for oncogenicity studies based on OPPTS 870.4200 Guidelines.

7. Sacrifice and Pathology

After 12 months, 10 randomly selected mice/sex/group were sacrificed. All surviving animals were sacrificed after 18 months. All mice euthanized *in extremis*, found dead, or sacrificed on schedule were killed by carbon dioxide inhalation and examined grossly. The CHECKED (X) tissues from all groups were collected for histopathological examination. Bone marrow smears were prepared at scheduled necropsies only. The (XX) organs from all animals were weighed.

All tissues from the control and high-dose animals were examined microscopically. In addition, bone with bone marrow, kidney, liver, lung, and spleen were examined microscopically from all males and females and adrenal gland, eye, testis, and thyroid were examined microscopically from all males in the low- and mid-dose groups.

56

Lesions were graded as trace, mild, moderate, or severe (1-4, respectively). A formal peer review was conducted on the histopathologic findings.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
	Oral tissue	XX	Heart*	X	Periph. nerve*
XX	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	XX	Pituitary*
X	Stomach*	XX	Spleen*	X	Eye and optic nerve, left*
X	Duodenum*	XX	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+		Lacrimal/Harderian glands
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*+	X	Parathyroids*
XX	Liver*+	X	Epididymides	X	Thyroids*
XX	Gall bladder*	XX	Prostate		
X	Pancreas*	X	Seminal vesicle		OTHER
			Coagulating gland	X	Bone*
	RESPIRATORY	XX	Ovaries*	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin* and subcutis
XX	Lung*	X	Cervix		Mediastinal tissue
	Nose		Oviduct		Mesenteric tissue
	Pharynx	X	Vagina	X	All gross lesions and masses*

* Required for oncogenicity studies based on OPPTS 870.4200 Guidelines.
+ Organ weight required in oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

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. Throughout the remainder of the study, the incidences of decreased defecation were similar between the control and high-dose groups. No other treatment-related clinical signs were seen in males or females during the daily or weekly examinations. Common findings in treated and control animals of both sexes included scabbing and hair loss. The incidences of palpable swellings or masses were similar between the treated and control groups of both sexes.

2. Mortality

The percent survival at selected times during the study is given in Table 2. No significant treatment-related trends or differences in survival were noted for treated males or females compared to the control groups.

57

TABLE 2. Survival of male and female mice fed acetamiprid for 78 weeks [No. alive (%)]

Weeks of study	Dietary concentration (ppm)			
	0	130	400	1200
Males (n = 50)				
Week 52	48 (96)	49 (98)	48 (96)	45 (90)
Week 78	39 (78)	42 (84)	38 (76)	39 (78)
Females (n = 50)				
Week 52	47 (94)	49 (98)	45 (90)	47 (94)
Week 78	38 (76)	43 (86)	39 (78)	43 86)

Data calculated by reviewer from Table 1, pp. 44-51, MRID 44988428.

B. BODY WEIGHT

The mean body weights and body weight gains of male and female mice are summarized in Table 3. High-dose males and females had significantly ($p \leq 0.01$) lower absolute body weights as compared with that of the controls throughout the study. Absolute body weights of the high-dose males and females were 83-93% and 82-91%, respectively, of the control levels during the study. The most pronounced effect on body weights occurred during the first year of the study when weight gain by the high-dose males and females was 50% and 55%, respectively, of the controls. During the last 6 months of the study, weight gain by the high-dose males and females was comparable to that of the controls. Body weights of the mid-dose groups were slightly less than that of the controls throughout the study with statistical significance ($p \leq 0.05$ or 0.01) attained for males at most timepoints but for females on only two occasions. Weight gain by the mid-dose males was significantly ($p \leq 0.01$) less than that of the control for the week 0-13 interval. Weight gain by the mid-dose females was similar to the controls throughout the study. Body weights and body weight gains for the low-dose group were not affected by treatment.

TABLE 3. Selected group mean body weights and body weight gains in male and female mice fed acetamiprid for 78 weeks (g)

Study Interval	Dietary concentration (ppm)			
	0	130	400	1200
Males				
Week 1	28	28	28	26** (93)[a]
Week 13	34	34	33** (97)	30** (88)
Week 24	36	36	35* (97)	30** (83)
Week 52	37	38	36	32** (86)
Week 64	38	38	37	32** (84)
Week 78	38	38	36	32** (84)
Wt. gain 0-1	1	1	1	0**
Wt. gain 0-13	7	7	6** (86)	4** (57)
Wt. gain 0-48	10	11	9	5** (50)
Wt. gain 48-78	0	0	1	0
Wt. gain 0-78	11	11	10	6** (55)
Females				
Week 1	23	23	23	21** (91)
Week 13	28	28	28	25** (89)
Week 24	31	31	30	26** (84)
Week 52	33	33	32	27** (82)
Week 64	34	34	32	28** (82)
Week 78	34	34	33	28** (82)
Wt. gain 0-1	1	0**	2	0**
Wt. gain 0-13	7	6**	7	3** (43)
Wt. gain 0-48	11	10	11	6** (55)
Wt. gain 48-78	2	1	2	1
Wt. gain 0-78	12	11	12	7** (58)

Data taken from Table 5, pp. 72-77, MRID 44988428.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *p≤0.05, **p≤0.01.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Selected food consumption values are given in Table 4. Food consumption by the high-dose males and females was less than that of the controls throughout the study with statistical significance ($p \leq 0.05$ or 0.01) reached at most intervals. Food consumption by the high-dose groups was <85% of the control levels during weeks 1-13, but increased steadily during the remainder of the study. By the end of the study, food consumption by the high-dose males and females was 90% and 94%, respectively, of the control levels. Food consumption by the low- and mid-dose groups was occasionally less than or greater than that of the controls, however, no consistent trends were apparent.

59

TABLE 4: Selected food consumption values for male and female mice fed acetamiprid for 78 weeks (g/animal/day)				
Study interval	0 ppm	130 ppm	400 ppm	1200 ppm
Males				
Week 1	5.5	5.5	5.4	4.4** (80)[a]
Week 13	5.8	5.6	5.9	4.8** (83)
Week 24	5.6	5.6	5.8*	4.9** (88)
Week 52	5.4	5.4	5.4	4.9** (91)
Week 78	5.1	4.9	4.9	4.6** (90)
Females				
Week 1	5.0	5.2	5.1	4.1** (82)
Week 13	5.9	6.0	5.6*	4.5** (76)
Week 24	5.6	6.0**	5.7	4.8** (86)
Week 52	5.4	5.6	5.5	4.9** (91)
Week 78	5.0	5.1	4.9	4.7

Data taken from Table 7, pp. 82-85, MRID 44988428.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *p≤0.05; p≤0.01.

2. Compound consumption

The compound consumption was calculated from the food consumption and body weight data. The results are given in Table 1.

3. Food efficiency

Weekly food efficiencies were calculated for the first 16 weeks of the study. For the high-dose groups, food efficiencies were significantly ($p \leq 0.01$) less than the control values for males at weeks 1-4 and for 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. Food efficiency values for the high-dose groups after week 4 and for the low- and mid-dose groups at all remaining calculated intervals were somewhat variable with no apparent trends.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

D. BLOOD WORK

1. Hematology

No significant differences were seen in any hematology parameter between the treated and control groups of either sex at 12 months or at termination.

60

E. SACRIFICE AND PATHOLOGY

1. Organ weight

At interim sacrifice, terminal body weights of the high-dose males and females were significantly ($p \leq 0.01$) less than that of controls. In high-dose males significant ($p \leq 0.05$ or 0.01) differences in absolute or relative organ weights included decreased absolute and relative (to brain weight) heart and kidney weights, increased relative (to body weight) pituitary weight, and decreased absolute prostate weight. The only significance ($p \leq 0.01$) difference observed in high-dose females was a decreased absolute kidney weight. In addition, low-dose males had significantly ($p \leq 0.05$) increased pituitary weights relative to body weight as compared with the controls.

Selected organ weight data from terminal sacrifice are given in Table 5. Final body weights of the high-dose males and females were significantly ($p \leq 0.01$) less than that of the controls. The high-dose males and females had significantly ($p \leq 0.05$ or 0.01) decreased absolute brain and increased brain/body weight ratios, decreased absolute kidney weights, and increased liver/body weight ratios. In addition, high-dose males had significantly ($p \leq 0.05$ or 0.01) increased heart-, testes-, and spleen-to-body weight ratios, and decreased absolute pituitary and prostate weights. Mid-dose males had significantly ($p \leq 0.05$) decreased absolute kidney weights and increased spleen/body weight ratios. For mid- and high-dose females, absolute and relative (to both body and brain weights) adrenal weights were significantly ($p \leq 0.05$ or 0.01) less than those of the controls. High-dose females also had significantly ($p \leq 0.05$) decreased absolute heart weight, increased kidney/body weight ratios, and decreased ovary weights. Liver/body weight ratios were significantly ($p \leq 0.01$) increased for the mid-dose females as compared to that of the controls.

TABLE 5. Group mean organ and final body weights in male and female mice fed acetamiprid 78 weeks

Organ	0 ppm	130 ppm	400 ppm	1200 ppm
Males				
Final body wt. (g)	38	38	36	32**
Brain wt. (g)	0.52	0.50	0.52	0.49**
Liver weights				
absolute (g)	2.24	2.18	2.25	2.23
relative to body wt. (%)	5.93	5.78	6.25	6.90**
Kidney weights				
absolute (g)	0.90	0.87	0.82*	0.74**
relative to body wt. (%)	2.39	2.32	2.27	2.35
Spleen weight				
absolute (g)	0.11	0.13	0.13	0.13
relative to body wt. (%)	2.80	3.39	3.80*	4.21*
Females				
Final body wt. (g)	33	34	32	28**
Brain wt. (g)	0.53	0.53	0.53	0.51**
Liver weights				
absolute (g)	1.97	2.09	2.13	1.95
relative to body wt. (%)	5.92	6.20	6.57**	7.01**
Kidney weights				
absolute (g)	0.58	0.60	0.59	0.53*
relative to body wt. (%)	1.75	1.78	1.83	1.90*
Adrenal weight				
absolute (mg)	14.3	14.6	12.3*	10.1**
relative to body wt. ($\%x10^2$)	4.35	4.34	3.82*	3.59**

Data taken from Table 12, pp. 119-126, MRID 44988428.
Significantly different from the control: *p≤0.05; **p≤0.01.

2. Gross pathology

No treatment-related lesions were observed in males or females at gross necropsy. Findings common to treated and control animals of both sexes included scabs on the skin, corneal opacity, and discolorations on the liver and kidney.

3. Microscopic pathology

a) Non-neoplastic

At interim sacrifice centrilobular hepatocellular hypertrophy was observed in 8/10 high-dose males (severity = 1.50) and 8/10 high-dose females (severity = 1.13) compared with 0/10 of the controls. Myeloid hyperplasia in the bone marrow of the femur was observed in 0/10, 1/10, 2/10, and 4/10 (p≤0.05) males in the control, low-, mid-, and high-dose groups, respectively.

For animals that were sacrificed at study termination, centrilobular hepatocellular hypertrophy was observed in 23/39 high-dose males (p≤0.01; severity = 1.17), in 3/38 mid-dose females (n.s.; severity = 1.00), and in 16/43 high-dose females (p≤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

62

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 6. Incidences of selected non-neoplastic microscopic findings in male and female mice fed acetamiprid 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/e	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*

Data taken from Table 13, pp. 151-196, MRID 44988428.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.
n/e = not examined

63

b) Neoplastic

A summary of common neoplasms seen at terminal sacrifice in this study is given in Table 7. 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.

TABLE 7. Neoplastic findings in male and female mice fed acetamiprid 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%)				

Data taken from Tables 13 & 14, pp. 127-150, 257-272, MRID 44988428.
[a]Data taken from Tables 3 and 4, pp. 9-20, MRID 45245305; includes studies conducted after the current study was completed.
[b]Number of animals with lesion (% of animals examined with lesion)
[c]Includes animals sacrificed at 12 months and animals which died prior to 12 months.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that the administration of up to 1200 ppm acetamiprid in the diet of mice for up to 78 weeks resulted in no significant increases in the incidences of any type of neoplastic lesions compared to control animals.

Treatment at 1200 ppm resulted in decreases in defecation, body weights, and food consumption. Decreased body weights were also observed at 400 ppm. Liver-to-body

weight ratios were increased in males and females at 1200 ppm and in females at 400 ppm. Hepatocellular hypertrophy was seen in high-dose males and females at 12 and 18 months. A low incidence of hepatocellular hypertrophy was also seen in males and females at 400 ppm. The no-observed-effect level (NOEL) was 130 ppm.

B. REVIEWER'S DISCUSSION

Survival of male and female mice was not affected by treatment with acetamiprid. During the first year of the study, lower body weight gains and decreased defecation observed in the high-dose group corresponded to reduced food consumption by these animals.

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. Mid-dose males also had a reduction in body weight gain when compared to the control group during the first 90 days (86% of the control value, 9 < 0.01); however, this reduction did not continue and by the end of the first year, mean body weight gain in this group was similar to the control value.

The decrease in food consumption may have been due to a lack of palatability of the diet to the animals (<85% of the control levels during weeks 1-13 at the high dose, ($p \leq 0.01$)). However, because of lower food efficiencies for the first few weeks, systemic toxicity may also have accounted for the lower body weight gains by the high-dose males and females. Lower body weight gain at the beginning of the study by the mid-dose males was not accompanied by differences in food consumption, but did result in lower food efficiency and is, therefore, considered a result of systemic toxicity. In the mid-dose females, sporadic differences in absolute body weights with no corresponding effects on body weight gain or food consumption are not considered biologically significant or treatment-related.

No treatment-related changes were seen in hematology parameters and gross necropsy was unremarkable. At both interim and terminal sacrifice, differences in organ weights of the high-dose groups as compared with the controls were attributed to lower final body weights of the treated animals.

Microscopic lesions were noted in several organs. Centrilobular hepatocellular hypertrophy was observed at an increased incidence rate in high-dose males and females at both interim and terminal sacrifice. This lesion is likely due to enzyme induction, did not increase in severity with dose or time, and is not considered an adverse effect. The incidence rate of myeloid hyperplasia in the bone marrow was slightly increased in high-dose males at interim sacrifice and slightly or significantly increased in all treated male and female groups at terminal sacrifice. Even though myeloid hyperplasia was not observed in any of the control animals, the rate in the treated groups did not increase with dose. Therefore, the relationship to treatment is uncertain at this time. In males at terminal sacrifice amyloidosis was observed in numerous organs of the high-dose animals and in several organs of the mid-dose animals. The mechanism by which the test article may cause diffuse amyloidosis is unknown, but this lesion is considered by the reviewer to be treatment-related.

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 of male and female mice for up to 78 weeks did not result in a significant increase in the incidence of neoplastic lesions in this study. The animals were adequately dosed as evidenced by decreases in body weight gains and microscopic lesions.

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. Dosing was considered adequate based on decreased body weight gain and microscopic lesions in the high-dose group.

C. STUDY DEFICIENCIES

No deficiencies were noted for this study.

DER #3

Acetamiprid: 1-Year Feeding Study in Dogs
Nippon Soda Company. 1998. MRID No. 44651846

Reviewer: Gordon Cockell, Date June 27, 2001

STUDY TYPE: Oral 1-Year Dog Study, Dietary; OPPTS 870.4100 [§82-1]; OECD 452.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.57%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Auletta, C.S. (1998) A chronic (12-month) oral toxicity study of NI-25 in the dog via dietary administration. Pharmaco LSR, Inc. East Millstone, NJ. Study no. 92-3117. April 29, 1998. MRID No. 44651846. Unpublished.

SPONSOR: Nippon Soda, Tokyo, Japan

EXECUTIVE SUMMARY: 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).**

This study is classified as acceptable and it satisfies the guideline requirement for a 1-year oral toxicity study (870.4100; OECD 452) in the dog.

COMPLIANCE: Signed and dated GLP, QA and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: NI-25 (Acetamiprid)

Description:	pale yellow powder
Lot/Batch #:	NNI-03
Purity:	99.57 % a.i.
Compound Stability:	Stable for 4years and 3 months in the dark at -20°C
CAS #:	135410-20-7

2. Test animals:

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Approximately six months, males 9.3 kg (7.6-10.3), females 8.3 kg (7.6-10.1)	
Source:	Marshall Farms, U.S.A., Inc.	
Housing:	Individual, in elevated metal grid cages. Animals were provided with exercise according to Animal Welfare Standards, following Bio/Dynamics Standard Operating Procedures	
Diet:	Standard laboratory diet (Purina Certified Canine Meal Diet #5007), 400 g/animal/day, available for 22 hours per day.	
Water:	Tap water, available *ad libitum*	
Environmental conditions:	**Temperature:**	19-28°C
	Humidity:	31-90%
	Air changes:	Not stated
	Photoperiod:	12 hour light/dark cycle (7 am - 7 pm via automatic timer)
Acclimation period:	Approximately 4 weeks	

B. STUDY DESIGN:

1. In life dates - Start: January 8, 1993 **End:** January 11, 1994

2. Animal assignment: Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw/day)	# Male	# Female
Control	0	0	4	4
Low	240	9/9	4	4
Mid	600	20/21	4	4
High	1500	55/61	4	4

3. Diet preparation and analysis: Appropriate amounts of test substance were mixed with the Certified Diets to achieve the desired concentrations. Fresh diets were prepared weekly. Homogeneity analyses were conducted on a mock batch of the low concentration diet prior to initiation of dosing. Three samples were taken from the top, middle and bottom sections of the mock dietary batch. Stability of the test substance in the dietary mixture was demonstrated in the 4-week range-finding study. In that study, stability was demonstrated after storage of test diets at room temperature for 15 days. Concentration

analysis of test diets was conducted weekly for the first 4 weeks and monthly thereafter to ensure that the diets were prepared at their intended concentrations.

Results - **Homogeneity Analysis:** The homogeneity of the test diet ranged from -7% to +7% of the mean concentration, for samples taken from the top, middle and bottom of the mixture. The mean concentration (% of nominal ± standard deviation) of the 240 ppm mock diet was 100 ± 4.67%.

Stability Analysis: In the range-finding toxicity study in dogs, NI-25 was found to be stable in test diets when stored at room temperature over a period of 15 days. After 15 days of storage at room temperature, concentrations ranged from 89.6% to 103% of nominal concentrations.

Concentration Analysis: The mean test material concentration in prepared diets were 98.3 ± 6.82%, 99.3 ± 5.59% and 98.1 ± 4.83% of nominal concentrations for the 240, 600 and 1500 ppm dose groups, respectively.

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

4. Statistics - Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison method if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric methods were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a non-parametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

   A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the non-parametric case, Jonckheere's test for monotonic trend was used.

   The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other tests were conducted at the 5% and 1%, two-sided risk level.

C. METHODS:

1. Observations: Animals were inspected at least twice daily for signs of toxicity and mortality. Detailed physical examinations were conducted weekly

2. Body weight: Animals were weighed twice pretest, weekly during the treatment period and at study termination after fasting.

3. Food consumption and test article intake: Food consumption for each animal was measured and recorded daily (seven days per week) and reported as weekly means. Nominal test article intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption and body weight data.

4. **Ophthalmoscopic examination**: All animals were examined pretest and at study termination. Eyelids, lacrimal apparatus and conjunctiva were examined grossly; cornea, anterior chamber, iris, lens, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy. Eyes were examined after installation of Mydriafair 1% or Opticyl 1%.

5. **Haematology & clinical chemistry**: Blood was collected from all animals pretest, at week 13 (hematology only), and at 6 and 12 months for haematology and clinical biochemical analysis. Blood was obtained from unanesthetized animals via the jugular vein. Animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. Haematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
X	Plasma Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Serum alanine amino-transferase (also SGPT)*		Phospholipids
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. **Urinalysis**: Urine was collected from water-deprived animals (approximately 2 hours) pretest and at 3, 6 and 12 months. Urine volume was measured over a 16-hour interval. Animals were water-deprived for this interval. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.1350

7. Sacrifice and Pathology: Gross postmortem examinations were conducted on all animals and the CHECKED (X) tissues were collected for histopathological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph. nerve (sciatic)*
X	Esophagus*	X	Sternum with bone marrow*	X	Spinal cord (3 levels)[T]
X	Stomach*	X	Lymph nodes (mesenteric, submandibular)*	X	Pituitary*
X	Duodenum*			X	Eyes (optic n.)[T]
X	Jejunum*	X	Spleen*		
X	Ileum*	X	Thymus*		GLANDULAR
X	Cecum*			XX	Adrenal gland*
X	Colon*		UROGENITAL		Lacrimal gland[T]
X	Rectum*	XX	Kidneys*+	X	Mammary gland[T]
XX	Liver*+	X	Urinary bladder*	XX	Parathyroids*++
X	Gall bladder*	XX	Testes*+	XX	Thyroids*++
X	Pancreas*	XX	Epididymides		
		X	Prostate		OTHER
	RESPIRATORY		Seminal vesicle	X	Skeletal muscle
X	Trachea*	XX	Ovaries	X	Skin
X	Lung*	X	Oviducts	X	All gross lesions and masses*
	Nose		Vagina		
	Pharynx	X	Uterus*		
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. Clinical signs of toxicity and physical examinations - No noteworthy observations were made during the weekly physical examinations and no clinical signs of toxicity were recorded during routine observations of the animals. One high dose male had a brief, transient convulsion at the time of feeding on study day 95. The study author reported that this animal had stiffened, twitching limbs and an arched back with the head raised and eyes rolled back. The episode lasted for approximately 3 minutes, after which time the animal appeared normal. No subsequent observations of this nature were made, and the occurrence of a single mild convulsion at that time in the study was considered to be a spurious finding.

2. Mortality - All animals survived throughout the study.

B. Body weight and weight gain: High dose animals lost weight during the first few weeks of the study. Significantly lower cumulative body weight gain was recorded for females at week 2 and for males at week 7. No other statistically significant differences were recorded. Following these initial decreases, body weight stabilized and body weight gain was similar to control values, except in high-dose females, where a mean loss of 0.1 kg was recorded for the 52 week study period. Final body

weights were 16% and 20% lower than controls for high dose males and females, respectively. Body weight and body weight gain were unaffected among low- and mid-dose animals.

TABLE 2: Body weight and body weight gain

Dose rate (ppm)	Body Weights (g)						Total Weight Gain	
	Week 0	Week 1	Week 3	Week 13	Week 26	Week 52	kg	% of control
Male								
0	9.3±0.8	9.2±0.7	9.7±0.4	10.5±0.5	11.1±0.6	11.9±1.2	2.7±2.0	100
240	9.2±1.2	9.5±1.1	9.5±1.5	9.7±1.8	10.2±1.6	11.6±1.4	2.3±1.2	85
600	9.3±0.6	9.4±0.6	9.6±0.7	10.2±1.6	10.9±1.3	11.5±1.9	2.2±1.5	81
1500	9.3±0.5	9.4±0.4	8.8±0.6	9.0±0.7	9.4±0.6	10.0±0.8	0.6±0.6	22
Female								
0	8.3±0.8	8.2±0.7	8.0±0.7	7.8±1.3	8.8±1.7	10.2±2.5	1.9±2.5	100
240	8.4±1.2	8.5±1.2	8.8±1.0	9.1±1.1	10.1±1.2	11.6±1.7	3.2±1.1	168
600	8.3±0.4	8.1±0.8	8.2±0.7	8.6±0.7	9.4±0.4	10.2±0.6	1.9±0.7	100
1500	8.3±0.3	7.8±0.6	7.4±0.8	7.6±0.7	7.8±0.8	8.2±0.5	-0.1±0.5	-5

Data extracted from pages65-87 of the study report

C. **Food consumption and compound intake:**

1. **Food consumption** - Significantly decreased food consumption was recorded among high-dose males and females during the first two weeks of the study. From week 3 until the end of the study period, food consumption was similar to controls for all treated male groups and similar to or slightly lower than controls for treated female groups.

2. **Compound consumption** - Mean compound consumption is shown in Table 3, below.

TABLE 3: Mean test article intake (mg/kg bw/day)

Dietary concentration (ppm)	0	240	600	1500
Males	0	9	20	55
Females	0	9	21	61

D. **Ophthalmoscopic examinations:** There were no observations at the terminal ophthalmoscopic examination that were attributed to treatment with acetamiprid.

E. **Blood analyses:**

1. **Haematology** - There were no changes in hematological parameters that could be attributed to treatment with acetamiprid.

2. **Clinical Chemistry** - There were no changes in clinical biochemistry parameters that could be attributed to treatment with acetamiprid. Slightly lower inorganic phosphorus values were recorded in high-dose male and female dogs, however the difference in females was not statistically significant, and in the absence of any corresponding pathology, this observation was not considered to be biologically significant.

F. Urinalysis: There were no significant differences in urinalysis parameters between control and treated animals.

G. Sacrifice and Pathology:

1. Organ weight: There were no differences in absolute organ weights or organ-to-body weight ratios in any of the treated male or female groups relative to controls. Kidney-to-brain weight was significantly decreased among high-dose males, and liver-to-brain weight was significantly decreased at all doses. The study author attributed the differences among high-dose animals to the observed reduction in body weight at that dose. In the absence of morphological changes in the liver, the slight changes in liver-to-brain weight among low- and mid-dose animals were not considered to be toxicologically relevant.

Table 4: Select organ weights and organ weight ratios for male dogs

	Control	240 ppm	600 ppm	1500 ppm
Terminal body weight (kg)	11.8±1.3	11.2±1.5	11.3±1.8	9.6±0.6
Absolute brain weight (g)	77.9±5.5	84.6±3.0	83.7±5.5	84.2±5.7
Absolute kidney weight (g)	57.6±6.8	51.9±9.0	60.6±11.9	44.7±3.7
Kidney-to-brain weight	7.38±0.6	6.15±1.2	7.22±1.3	5.30±0.3*
Absolute liver weight (g)	327.2±23	299.5±38	291.9±44	278.0±23
Liver-to-brain weight	4.20±0.1	3.54±0.4*	3.48±0.4*	3.31±0.3**

Data obtained from page 140 of the study report
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

2. Gross pathology: There were no macroscopic changes observed at necropsy that were attributed to treatment with acetamiprid.

3. Microscopic pathology: There were no microscopic changes that were attributed to treatment with acetamiprid.

III. DISCUSSION

A. Investigators' conclusions: "Based on the initial body weight losses and decreased body weight gains during the study in males and females receiving the highest dietary concentration (1500 ppm) of NI-25, the no observed effect level (NOEL) for dietary administration of this material to dogs for one year under conditions of this study was 600 ppm (20 and 21 mg/kg/day for males and females, respectively)."

B. Reviewer comments: In a one-year oral toxicity study in Beagle dogs, acetamiprid was administered in the diet at nominal concentrations of 0, 240, 600 or 1500 ppm, equal to daily average intakes over the study period of 0, 9, 20 and 55 mg/kg bw/day in males and 0, 9, 21 and 61 mg/kg bw/day in females.

Treatment with acetamiprid had no effect on mortality, clinical signs of toxicity, ophthalmoscopic examinations, 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).**

C. Study deficiencies: None.

DER #4

Acetamiprid: 2-Generation Reproduction Study in Rats
Nippon Soda. 1999. MRID No. 44988430

Reviewer: Gordon Cockell, Date March 21, 2001

STUDY TYPE: Multigeneration Reproduction Study - rat - OPPTS 870.3800; OECD 416.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.9%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Trutter, J.A. (1999) Two-Generation Reproduction Study with NI-25 in Rats (Reproduction and Fertility Effects). Covance Laboratories, Inc., Vienna, VA. Laboratory Study Identification Covance 6840-108, November 13, 1999. MRID 44988430. Unpublished

SPONSOR: Nippon Soda Co., Ltd., Tokyo, Japan

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 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 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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** NI-25 (Acetamiprid)

Description:	technical active ingredient, white powder
Lot/Batch #:	NGL-30
Purity:	99.9 % a.i.
Compound Stability:	"Expiration date March 2000"
CAS #:	135410-20-7

2. **Test animals:**

Species:	Rat	
Strain:	Crl:CD BR (IGS) Sprague-Dawley	
Age at study initiation:	(P) 6 wks; (F_1) 3 wks	
Wt. at study initiation:	(P) Males: 161-193 g; Females: 118-154 g (F_1) Males: 44-130 g; Females: 43-111 g	
Source:	Charles River Laboratories, Inc., Raleigh, N.C.	
Housing:	Individual, in suspended wire mesh cages; one male and one female per cage during breeding until confirmed mating; on day 20 of presumed gestation, females were placed in polycarbonate nesting boxes for the parturition and lactation periods	
Diet:	Certified Rodent Diet #5002 (PMI Feeds, Inc.), *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18-26 °C
	Humidity:	30-70 %
	Air changes:	10 or greater / hr
	Photoperiod:	12 hrs dark / 12 hrs light
Acclimation period:	approximately 1 week	

B. PROCEDURES AND STUDY DESIGN

1. **Mating procedure:** After at least 10 weeks of treatment, one male and one female were housed together during the breeding period. For the F1 generation, care was taken to avoid sibling matings. Once mating occurred, the animals were returned to their individual cages. Each pair was given a maximum of 14 days to mate. Females that showed no evidence of mating were placed in nesting boxes. Pregnant females were placed in nesting boxes on day 20 of presumed gestation, for the duration of the parturition and lactation periods. Daily examinations were conducted to detect the presence of a copulatory plug or vaginal sperm. The day of observation of a plug or sperm was designated as gestation day 0. Day 0 of lactation was established at the completion of delivery, or in case of prolonged delivery, approximately 24 hours after the first observation of littering. Day 0 of lactation marked the end of the gestation period.

2. **Study schedule:** The parental animals were given test diets for at least 10 weeks before mating. The F_1 parental animals were not mated until at least 10 weeks after selection from the F_1 litters. Selection of the parents for the F_1 generation was done immediately after weaning at 3 weeks, hence the F_1 animals were approximately 13 weeks of age at the time of mating.

3. **Animal assignment:** Parental animals were randomly assigned to test groups identified in Table 1.

TABLE 1 Animal Assignment

Test Group	Dose in Diet * (ppm)	Animals/group			
		P Males	P Females	F_1 Males	F_1 Females
Control	0	26	26	26	26
Low	100	26	26	26	26
Mid	280	26	26	26	26
High	800	26	26	26	26

* Diets were administered from the beginning of the study until sacrifice

4. **Dose selection rationale**: The high dose was selected based on all the data available at the time of study design, including the results of other toxicity studies. The high dose was selected with the expectation that it would induce some development and/or maternal toxicity, but not more than 10% maternal mortality. The intermediate dose was selected to induce minimal observable toxic effects, and the low dose was expected to produce no evidence of maternal or developmental toxicity.

5. **Dosage preparation and analysis:** Diet formulations were prepared at least weekly by mixing appropriate amounts of test substance with approximately 200 g of feed until a homogeneous mixture was obtained this was then mixed with the required amount of feed to achieve the proper dietary concentration. Test diets were stored at room temperature and protected from light until dispensed for dosing. Prior to the start of the study, stability of the test substance in the samples from the low- and high-dose diet was evaluated for a period of 0, 7, 10 and 14 days at room temperature. Homogeneity of the low- and high-dose diets (top, middle, and bottom of the diet preparations) was evaluated prior to initiation of treatment and at week 11. During the study, routine concentration analyses were performed in duplicate on samples taken at week 1, 4, 13 and 26.

Results:

a) Homogeneity Analysis: Homogeneity analyses indicated that the test material was evenly mixed throughout the test diets. Samples taken on day 0 from the top, middle and bottom of the 100 ppm diet ranged from 97-105% of the target concentration, with a mean value of 102%. Samples taken on day 0 from the top, middle and bottom of the 800 ppm diet ranged from 98-103% of the target concentration, with a mean value of 100%. At week 11, the mean concentration at 100 ppm was 97% (92-104) and at 800 ppm it was 98% (93-104).

b) Stability Analysis: Stability analyses conducted on samples stored at room temperature for 0, 7, 10 or 14 days indicated that the diet preparations were stable for 14 days. All day 14 values were within 8% of the initial concentration.

c) Concentration Analysis: Routine concentration analysis showed that the diet preparations were within 10% of the target concentrations, except for one sample of the 100 ppm diet at week 26, which was approximately 88% of the target. The mean concentration of the duplicate samples of the 100 ppm diet at week 26 was 92%. Overall, the mean concentrations of the 100, 280 and 800 ppm diets were 99, 99 and 97%, respectively.

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

C. OBSERVATIONS

1. **Parental animals:** Observations and the schedule for those observations are summarized from the report.

Mortality and clinical observations: Mortality and moribundity checks were conducted twice daily. Clinical observations were recorded daily (by exception, noting only those animals for which observations were remarkable). A thorough physical examination was conducted at each weighing interval.

Estrous cycle determinations: Beginning 3 weeks prior to cohabitation and throughout the mating period, a daily vaginal smear from each female was assessed for the stage of estrus. Estrous cycle determinations continued until confirmation of mating occurred or the mating period ended. For non confirmed-mated females, estrous cycle determination continued until termination (completion of the lactation phase for females that proved to be pregnant).

Mating procedures: After at least 10 weeks of treatment, one female was placed into the breeding cage of one randomly selected male from the same treatment group; sibling matings were avoided in the F_1 generation. Each pair was allowed a maximum of 14 days to mate. Once mating occurred, animals were returned to individual housing. Daily examinations were performed to detect the presence of a copulatory plug or vaginal sperm; the day this was recorded was designated gestation day 0. Day 0 of lactation was established at the completion of delivery and marked the end of the gestation period. Females for which no evidence of mating was observed were placed in nesting boxes.

Body weight: Males were weighed prior to treatment, weekly during treatment and at the time of necropsy. Females were weighed prior to treatment, weekly during the premating, mating and postweaning phases. Females were also weighed on gestation days 0, 7, 14 and 20 and on lactation days 0, 4, 7, 10, 14, 17 and 21 and at the time of necropsy.

Food consumption: Food consumption was recorded weekly during the premating period, however it was not recorded for either sex during the mating period nor for males after mating. For confirmed-mated females, food consumption was recorded on gestation days 0, 7, 14 and 20 and for those that delivered, on lactation days 0, 4, 7, 10 and 14. Food efficiency and test material consumption were calculated using the following formulas:

$$\text{Food efficiency} = \frac{\text{Average daily body weight gain}}{\text{Average daily food consumption}}$$

$$\text{Test material intake} = \text{concentration in diet (ppm)} \times \frac{\text{Average daily food consumption}}{\text{Average daily body weight gain}}$$

2. **Litter observations:** According to the report, the following litter observations (X) were made (see Table 2).

TABLE 2: F_1 / F_2 Litter Observations [a]

Observation	Time of observation (lactation day)					
	Day 0	Day 4[b]	Day 4[c]	Day 7	Day 14	Day 21
Number of live pups	X	X	X	X	X	X
Pup weight	X	X	X	X	X	X
Clinical observations	X	X	X	X	X	X
External alterations	X					
Number of dead pups	X					
Sex of each pup (M/F)	X					

[a] Data extracted from Covance report 6840-108
[b] Before standardization (culling)
[c] After standardization (culling)

Stillborn and liveborn (but found dead) pups were distinguished at necropsy by the presence/absence of milk in the stomach and the inability/ability of their lungs to float when placed in water. On day 4 postpartum, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible); culled pups were sacrificed by intraperitoneal injection of sodium pentobarbital and examined for cervical, thoracic or abdominal viscera abnormalities and gross lesions were preserved in 10% neutral-buffered formalin.

Developmental landmarks of pinna unfolding (beginning on day 1), upper incisor eruption (beginning on day 7) and eye opening (beginning on day 11) were evaluated until all pups within each litter were positive.

Litters were observed daily for signs of abnormal behaviour or ill health. Daily mortality records were maintained throughout the lactation period and the number of pups of each sex that were missing, found dead or sacrificed *in extremis*, with or without evidence of cannibalization, was recorded. Pups which died were examined externally and internally for cervical, thoracic or abdominal viscera abnormalities and were preserved in alcohol. Cannibalized pups were recorded as such and discarded without necropsy.

The F_2 pups were individually identified at birth and anogenital measurements were recorded on day 0.

Weaning and selection procedures: At the completion of weaning one pup/sex/litter was randomly selected and individually identified. When sufficient animals were not available to produce at least 26 rats/sex/group, additional animals were selected randomly to achieve this number. Records were maintained to avoid F_1 sibling matings.

Maturation phase: Clinical observations, mating and body weight and food consumption measurements were performed on all F_1 animals as described above for parental animals. Vaginal opening or cleavage of the balanopreputial gland was evaluated on an individual basis for the selected F_1 animals. Females

were examined for estrous cycle, mated and allowed to deliver their offspring as described for the F_0 generation, except that no F_2 animals were selected to produce an additional generation.

3. **Postmortem observations:**

1) **Parental animals:** All surviving parental animals were sacrificed following weaning of the pups. The animals were weighed, sacrificed by carbon dioxide inhalation and subject to a complete gross necropsy which included examination of the external body surfaces, all orifices, cranial cavity and cervical, thoracic and abdominal viscera. The uterus of each female was also examined for the presence of implantation sites. Tissues from control and high dose animals were examined microscopically. Reproductive organs of low and mid dose animals that failed to mate, sire or deliver healthy offspring were subject to histopathological examination.

The following tissues (X) were weighed and prepared for microscopic examination (XX):

XX	Ovaries (with oviducts)	XX	Testes (left testis preserved in Bouin's fixative)
XX	Uterus (with cervix)	XX	Epididymides (total and left cauda; right cauda weighed in conjunction with sperm count determinations)
XX	Vagina	XX	Coagulating gland
XX	Pituitary	XX	Prostate
XX	Lesions	XX	Seminal vesicles
X	Brain	X	Adrenal glands
X	Liver	X	Spleen
X	Kidneys	X	Thymus

2) **Offspring:** The F_1 offspring not selected as parental animals and all F_2 offspring were sacrificed following weaning at approximately 21 days of age. These animals were subjected to gross examinations of cervical, thoracic and abdominal viscera. Gross lesions were retained in 10% neutral buffered formalin. The brain, spleen and thymus were weighed.

D. DATA ANALYSIS

1. **Statistical analyses:** Mean parental and litter data of the treated groups were compared statistically to the data from the same sex of the control group using one way ANOVA. Levene's test was employed to analyse the homogeneity of variances (5%); if heterogeneous, analyses were conducted on rank-transformed data. Dunnett's t-test served as the *post hoc* group comparison test, at 5 and 1% two-tailed probability levels.

Pup weights, F_1 and F_2 landmark data (eye opening, incisor eruption and pinna unfolding), preputial separation, vaginal opening and anogenital distance were analysed using ANCOVA. The incidence of pregnant females and the viability and weaning indices were analysed using the Cochran-Armitage Test for Linear Trend followed by Fisher-Irwin Exact Test. Ovarian follicle and sperm analysis data was analysed using the Kruskal-Wallis nonparametric ANOVA test.

2. **Indices:**

Reproductive indices: The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Pregnancy rate: Number of females pregnant/number of females mated x 100
Male/female copulation index: Number of animals mated/number of animals paired x 100
Male fertility index: Number of males impregnating females/number of males mated x 100
Female fertility index: Number of females pregnant/number of females mated x 100
Gestation index: Number of females delivering live pups/number of females pregnant x 100

In addition to the above indices, the following information was reported for each group: Gestation duration (days), number of litters born and number of litters weaned.

Offspring viability indices: The following viability indices were calculated from lactation records of litters in the study:

Livebirth index: Number of pups born alive/number of pups delivered x 100
Viability index: Number of pups alive on day 4/number of pups born alive x 100
Weaning index: Number of pups alive at weaning/number of pups alive on day 4 x 100

3. **Historical control data:** Historical control data were provided for various parameters including food consumption during gestation, fertility, delivery and viability data as well as pup weights from parturition to weaning, and developmental landmark data from Covance Laboratories, Huntingdon Life Sciences, International Life Sciences Institute and WIL Research Laboratories.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs:

F_0 generation: One control male was found dead during week 2. All other males survived to scheduled termination and all females survived through the premating and gestation periods. There were no treatment-related clinical observations in either the males throughout the study or females during the premating and gestation periods.

One 100 ppm female was found dead on lactation day 18. One control female experienced total litter death on lactation day 5. Two 800 ppm litters were observed with weak pups on lactation days 11-17. None of the above observations were deemed to be related to treatment by the study author. The reviewer concurs with this interpretation.

F_1 generation: All males and females survived to the scheduled sacrifice. No treatment related clinical signs were observed in males throughout the study nor in females during the premating and gestation periods. Two 100 ppm females and five 800 ppm females experienced total litter deaths. There was no evidence of any treatment-related maternal clinical signs during the lactation period. Of the 5 total litter deaths reported at 800 ppm, 3 had pup observations of thin, pale and/or weak on the day or days preceding total litter death. These observations were also recorded for the two litters at 100 ppm prior to total litter death. The combined incidence of thin, pale and/or weak pups was 3, 4, 4 and 6 litters at 0, 100, 280 and 800 ppm, respectively. These observations occurred more frequently (i.e., on more days) as the dose level increased, particularly among those litters with total litter death, suggesting a possible relationship to treatment. The study author dismissed these findings as spurious.

2. Body weight and food consumption:

F_0 generation: Body weight was comparable between control, 100 and 280 ppm animals. Mean body weight was lower than controls among 800 ppm males and females throughout the study. After 10 weeks of treatment, male body weights were 94% of the controls and female body weights were 92% of control. Body weight gain was reduced at 800 ppm in both sexes for the duration of the premating period and in males at 280 ppm for the first 5 weeks of treatment. Mean body weight gain values for males and females at 800 ppm were 91 and 84% of control, respectively. The transient body weight effects among males only at 280 ppm were deemed to be treatment-related but not adverse.

Maternal body weight and body weight gain was comparable between control, 100 and 280 ppm females during the gestation and lactation periods. At 800 ppm, mean body weight and body weight gain was reduced compared to concurrent controls. During gestation, body weight and body weight gain was 92% of control and during lactation, maternal body weight was lower than control while body weight gain was increased relative to the control values, such that body weight by lactation day 21 was 96% of control.

Food consumption was decreased in a pattern similar to the above observations in body weight and body weight gain, although the data were more variable. There were no significant differences noted among animals treated at 100 ppm, nor in females at 280 ppm. Males in the 280 ppm group showed reduced food consumption during the first two weeks of the study. Mean total food consumption in males and females at 800 ppm were 94 and 91% of control, respectively. During the gestation and lactation periods, treatment-related reductions in mean food consumption was reported among 800 ppm females (80 and 88% for the gestation and lactation periods, respectively).

F_1 generation: Body weight and body weight gain were unaffected in males and females at 100 ppm and at 280 ppm in females. At 280 ppm, body weight gain was reduced in males over the first 3 weeks of the study. Body weight and body weight gain were reduced in males and females at 800 ppm. At week 13, body weights were 88 and 87% of controls in males and females, respectively. Body weight gain for these animals were 90 and 92% of control for the 13-week premating period. As in the F_0 generation, the transient body weight effects among 280 ppm males only were deemed to be treatment-related but not adverse.

During the gestation and lactation periods, there were no treatment-related changes in body weight or body weight gain at 100 and 280 ppm. At 800 ppm, body weight and body weight gain were decreased to 88 and 92% of control values, respectively during the gestation period. Mean maternal body weight was also lower than controls during the lactation period, however body weight gain was increased relative to controls during the latter half of the lactation period.

Food consumption was decreased in males and females at 800 ppm. Mean total food consumption for the 13 week premating period was 94 and 93% of controls for males and females, respectively. During the gestation period, food consumption was reduced at 800 ppm, to 87% of controls. During the lactation period, mean total food consumption was 91% of controls, however the difference was only statistically significant for the period of days 7-10 of lactation. There were no other differences that were attributed to treatment.

Reported body weight and selected food consumption results are summarized in Tables 4a and 4b.

TABLE 4a: F_0 generation Body Weight and Food Consumption - Pre-mating [a]

Observations/study week	Dose Group (ppm)			
	0	100	280	800
F0 Generation Male - Pre-mating				
Mean body weight (g)				
Week 0	176.8±6.8	177.0±7.1	177.0±9.0	176.7±7.3
Week 5	395.2±18.5	388.4±24.2	381.3±25.3	366.9±24.6**
Week 10	465.6±23.8	460.0±37.2	451.2±33.5	438.1±30.7**
Week 15	506.4±36.8	502.2±43.8	495.5±38.1	487.8±35.2
Week 20	522.5±41.7	516.5±41.9	514.0±34.8	501.3±37.9
Mean weight gain (g)				
Weeks 0-5	218.5±16.9	211.4±22.7	204.2±22.6*	190.2±19.1**
Weeks 0-10	288.8±23.3	283.0±36.2	274.1±31.6	261.4±25.3**
Weeks 10-20	56.9±23.0	56.5±18.1	62.8±17.3	63.2±17.4
Weeks 0-20	345.8±39.3	339.5±41.5	337.0±33.0	324.6±33.3
Mean food consumption (g/animal/day)				
Weeks 0-10	23.5±1.4	23.6±1.7	23.0±1.7	22.1±1.4**
F0 Generation Female - Pre-mating				
Mean body weight (g)				
Week 0	137.5±7.1	139.1±6.3	140.7±6.7	137.5±7.6
Week 5	238.2±20.8	235.3±12.5	242.8±14.0	220.2±17.5**
Week 10	269.7±21.8	265.0±17.5	271.7±16.8	248.4±24.8**
Mean weight gain (g)				
Weeks 0-5	100.7±17.4	96.2±10.4	102.1±11.6	82.7±13.7**
Weeks 0-10	132.2±17.7	125.8±15.3	131.0±14.9	110.9±21.8**
Mean food consumption (g/animal/day)				
Weeks 0-10	17.5±1.2	17.0±0.9	17.8±1.2	15.9±1.5**

[a] Data extracted from pages 84-110 of the study report
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

TABLE 4b: F_1 generation Body Weight and Food Consumption - Pre-mating [a]

Observations/study week	Dose Group (ppm)			
	0	100	280	800
F1 Generation Male - Pre-mating				
Mean body weight (g)				
Week 0	95.0±11.7	82.3±13.7**	91.0 ±14.8	74.6±16.9**
Week 5	372.8±26.4	364.7±22.1	363.0±30.1	321.6±36.4**
Week 10	494.1±35.3	485.1±29.2	480.3±39.4	433.6±39.7**
Week 15	538.4±41.4	532.7±38.9	525.8±52.8	482.8±53.2**
Week 20	566.8±44.2	561.3±42.7	554.9±53.9	509.7±56.7**
Week 25	588.6±55.7	583.8±51.2	568.0±49.9	534.3±63.5**

Mean weight gain (g)				
Weeks 0-5	277.8±19.7	282.4±18.2	272.0±22.2	247.0±25.4**
Weeks 0-13	434.0±36.4	438.2±39.5	418.7±47.4	391.3±43.5**
Weeks 13-25	62.5±28.3	62.2±23.1	63.6±35.6	68.3±53.5
Weeks 0-25	494.6±52.4	501.3±52.8	479.8±48.9	462.4±57.5
Mean food consumption (g/animal/day)				
Weeks 0-13	26.7±1.9	26.9±1.6	26.4±2.1	25.0±2.1**
F_1 Generation Female Parents				
Mean body weight (g)				
Week 0	85.7±10.0	79.3±10.5	82.1±12.8	66.4±13.2**
Week 5	229.8±17.8	228.6±17.9	222.2±17.4	195.1±20.7**
Week 13	292.7±22.7	294.6±26.1	284.1±20.9	255.7±26.8**
Mean weight gain (g)				
Weeks 0-5	144.1±13.8	149.3±17.4	140.0±14.5	128.7±19.5**
Weeks 0-13	207.9±19.6	214.8±26.5	203.0±20.7	190.9±23.1**
Mean food consumption (g/animal/day)				
Weeks 0-13	19.2±1.4	19.1±1.2	18.8±1.2	17.9±1.2**

[a] Data extracted from pages 203-226 of the study report
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.

3. **Test Substance Intake:** Based on food consumption, body weight and dietary analyses results, the doses expressed as mean daily mg test substance/kg body weight during the 10 week pre-mating period are presented in Table 5. The values for the F_0 generation are considered to be representative of the test substance intake for the entire study.

TABLE 5: Mean test substance intake during premating (mg/kg body weight/day) [a]

	Male			Female		
	100 ppm	280 ppm	800 ppm	100 ppm	280 ppm	800 ppm
F_0	6.5	17.9	51.0	7.6	21.7	60.1
F_1	7.5	21.0	63.3	8.4	23.8	72.6

[a] Data extracted from pages 35 and 46 of the study report

4. **Reproductive function:**

a. Estrous cycle length and periodicity: Vaginal smear data were not provided in this study. The estrous cycle determinations were similar among the females of both generations. There was no evidence of any treatment-related changes.

b. Sperm measures: Sperm motility, testicular and caudal epididymal sperm count and sperm morphology were not affected at 280 ppm in the F_0 generation, nor were motility and testicular sperm count affected at 800 ppm. Due to technical error, the epididymides were not suitable for evaluation in the F_0 males. A supplemental study was conducted to evaluate possible epididymal effects at 800 ppm among F_0 males. The results of the supplemental investigation indicated that treatment with acetamiprid at 800 ppm in the diet for 20 weeks did not affect testicular or epididymal sperm counts or sperm

morphology. No treatment-related effects were noted in sperm motility, testicular and caudal epididymal sperm counts and sperm morphology at 800 ppm in F_1 males.

5. **Reproductive performance:** There were no differences in reproductive performance between control and treated animals. Results for the parental animals are summarized from the report in Table 6.

TABLE 6: Reproductive Performance [a]

Observation	Dose Group (ppm)			
	Control	100	280	800
F_0 Generation				
Mean precoital interval (days)	3.1	3.4	3.9	3.1
MALES				
Mated	26	26	26	26
Fertile	26	25	25	26
Fertility not determined	0	1	1	0
Intercurrent deaths	1	0	0	0
FEMALES				
Number mated	26	26	26	26
Number fertile	26	25	25	26
Fertility not determined	0	1	1	0
Intercurrent deaths	0	1	0	0
Median gestation interval (days)	22.0	21.8	22.0	22.0
Number of litters	26	25	25	26
Male/female copulation index (% mated)	26/26 (100)	26/26 (100)	25/26 (96)	26/26 (100)
Male/female fertility index (% successfully mated)	26/26 (100)	25/26 (96)	25/25 (100)	26/26 (100)
F_1 Generation				
Mean precoital interval (days)	2.7	2.7	2.8	3.0
MALES				
Mated	26	26	26	26
Fertile	20	24	24	23
Fertility not determined	6	2	2	3
Intercurrent deaths	0	0	0	0
FEMALES				
Number mated	26	26	26	26
Number fertile	20	24	24	23
Fertility not determined	6	2	2	3
Intercurrent deaths	0	0	0	0
Median gestation interval (days)	22.1	22.0	22.1	21.8
Number of litters	20	24	24	23
Male/female copulation index (% mated)	23/26 (88)	26/26 (100)	25/26 (96)	26/26 (100)
Male/female fertility index (% successfully mated)	20/23 (87)	24/26 (92)	24/25 (96)	23/26 (88)

[a] Data extracted from pages 35, 46, 500-508 and 1049-1057 of the study report.

6. Parental postmortem results

a) Organ weights:

F_0 generation: Mean terminal body weight of 800 ppm females was significantly decreased, to 92% of the control value. The author reported associated changes in relative organ weights, attributable to the terminal body weight, including increased brain-to-body weight and decreased kidney-to-brain weight.
F_1 generation: Mean terminal body weights were significantly decreased at 800 ppm, to 90 and 88% of control for males and females, respectively. Absolute brain and kidney weight were reduced in 800 ppm females; absolute spleen, thymus, adrenal, testis and epididymis cauda weights were significantly decreased in 800 ppm males. Liver-to-body weight was increased in 800 ppm males and females; brain-, spleen-, and uterus-to-body weight were increased in 800 ppm females; and, spleen-to-brain weight was increased in 800 ppm females. The study author attributed all of these organ weight changes to the observed reduction in body weight among these animals.

Table 7: Selected organ weight data

	Males (ppm)				Females (ppm)			
	Control	100	280	800	Control	100	280	800
F_0 generation								
Terminal body weight SD	532.0 40.9	529.0 40.0	524.7 35.1	513.0 38.0	316.3 29.7	308.4 22.8	317.7 24.5	291.5* 23.7
Brain-to-body weight SD	0.413 0.033	0.421 0.031	0.428 0.035	0.428 0.036	0.628 0.051	0.667* 0.049	0.629 0.047	0.691* 0.058
Kidney-to-brain weight SD	1.600 0.165	1.567 0.113	1.526 0.151	1.569 0.176	1.169 0.091	1.149 0.145	1.159 0.091	1.083* 0.123
F_1 generation								
Terminal body weight SD	593.4 51.5	583.5 53.3	581.4 53.3	534.8* 60.7	344.1 27.7	344.7 32.7	336.5 25.8	302.8* 34.1
Absolute brain weight SD	2.30 0.11	2.25 0.14	2.22 0.13	2.07* 0.11	2.07 0.09	2.09 0.12	2.07 0.11	1.95* 0.10
Brain-to-body weight SD	0.390 0.039	0.388 0.040	0.384 0.033	0.391 0.044	0.604 0.058	0.611 0.053	0.620 0.057	0.648* 0.059
Absolute spleen weight SD	0.86 0.13	0.87 0.12	0.82 0.15	0.77* 0.09	0.60 0.08	0.65 0.11	0.64 0.07	0.64 0.11
Spleen-to-body weight SD	0.146 0.018	0.150 0.020	0.142 0.030	0.145 0.019	0.176 0.024	0.190 0.028	0.190 0.024	0.213* 0.042
Spleen-to-brain weight SD	0.376 0.053	0.388 0.055	0.370 0.060	0.374 0.047	0.292 0.038	0.312 0.050	0.307 0.032	0.329* 0.062
Uterus-to-body weight SD					0.198 0.053	0.202 0.044	0.204 0.061	0.241* 0.062
Absolute kidney weight SD	4.07 0.41	4.06 0.49	4.06 0.51	3.56* 0.38	2.55 0.34	2.50 0.23	2.47 0.19	2.28* 0.34
Liver-to-body weight SD	3.432 0.260	3.424 0.507	3.544 0.249	3.657* 0.318	3.685 0.255	3.707 0.396	3.676 0.219	3.975* 0.314
Absolute thymus weight SD	0.49 0.14	0.41 0.15	0.50 0.14	0.38* 0.12	0.36 0.10	0.42 0.17	0.37 0.17	0.33 0.08

Absolute adrenal weight SD	0.066 0.009	0.062 0.015	0.066 0.013	0.057* 0.009	0.092 0.090	0.079 0.033	0.088 0.027	0.074 0.022
Absolute testis weight SD	3.84 0.30	3.71 0.34	3.80 0.36	3.53* 0.55				
Epididymis, cauda (left) SD	0.40 0.05	0.37 0.06	0.40 0.06	0.34* 0.07				
Epididymis, cauda (right) SD	0.30 0.05	0.28 0.05	0.29 0.04	0.24* 0.04				

Data obtained from study pages 161-174 and 280-293
* Significantly different from control, $p < 0.05$

b) Pathology

1) **Macroscopic examination:** There were no treatment-related macroscopic observations among F_0 nor F_1 animals. Of the two F_0 animals that died prior to scheduled sacrifice, the control male had an enlarged, dark red liver and red fluid in the abdominal cavity and the 100 ppm female had a mass in the kidney and abdominal mesentery. There were no remarkable observations among the F_1 animals.

2) **Microscopic examination:** Microscopic examination did not reveal any treatment-related effects. The control male with the gross liver lesion noted above had intra abdominal hemorrhage as a result of a ruptured angiectatic liver lesion. The 100 ppm female noted above had a renal carcinoma that had spread to the abdominal cavity. In addition, two control F_0 males had severe or moderately severe degeneration of seminiferous tubules with severe hypospermia in the epididymides. This was attributed to ischemia resulting from vascular changes or torsion. Treatment had no effect on ovarian follicle count in either generation. Two 800 ppm F_1 males had severe or moderately severe degeneration of seminiferous tubules with severe hypospermia in the epididymides. As noted for the control F_0 males above, this was attributed to ischemia resulting from vascular changes or torsion. A mammary gland carcinoma was noted in one 800 ppm F_1 female. The author noted that this observation is unusual for a rat of this age and strain, however, this lone observation was not deemed to be related to treatment in the absence of any other changes in the reproductive tract in this animal and no other indications of changes in any other animals that might raise suspicion regarding its relationship to treatment. The reviewer concurs with this interpretation.

B. OFFSPRING

1. **Clinical signs:** Clinical signs observed in offspring during the lactation period are reported above, with the discussion of parental clinical signs.

2. **Natural delivery and litter data:** Pregnancy rate was not affected by treatment and there were no signs of abnormal gestation or delivery in either generation. The mean number of pups delivered and implantation sites per dam were not affected by treatment. Livebirth indices were similar between treated and control groups in both generations. In the F_1 pups, the viability indices were 95, 99, 98 and 96% for the 0, 100, 280 and 800 ppm groups, respectively. In the F_2 pups, the viability index was reduced at 800 ppm. The viability indices were 94, 90, 95 and 66% for the 0, 100, 280 and 800 ppm groups, respectively. Similarly, the weaning index was unaffected among F_1 pups and it was reduced among F_2 pups treated at 800 ppm. The F_1 weaning indices were 96, 99, 99 and 94% and the F_2 weaning indices were 98, 94, 97 and 73% for the 0, 100, 280 and 800 ppm groups, respectively. The mean litter size was reduced on lactation days 14 and 21 at 800 ppm in the F_1 litters. In the F_2 litters, the mean litter

size was reduced on lactation day 4 (precull), consistent with the reduced viability index at this dose. Postcull, the number of live pups was lower than controls at 800 ppm throughout the lactation period, however the difference was not statistically significant. Sex ratio was not affected by treatment. Mean litter size and viability (survival) results from pups during lactation are summarized from the report in Table 8.

TABLE 8: Litter parameters for F_1 and F_2 [a]

Observation	Dose Group (ppm)			
	Control	100	280	800
F_1 Generation				
Mean Implantation Sites	14.12	14.24	14.32	13.65
Number born live	350	342	330	329
Number born dead	1	4	6	9
Sex Ratio Day 0 (% ♂)	48.6	51.8	45.5	46.2
# Deaths Days 0-4 (%)	18 (5.1)	3 (0.9)	7 (2.1)	13 (4.0)
# Deaths Days 4-21 (%)	4 (1.1)	2 (0.6)	3 (0.9)	13 (4.0)
Mean litter size Day 0	13.46	13.68	13.20	12.65
Day 4[b]	12.77	13.56	12.92	12.15
Day 4[c]	7.81	8.00	7.76	7.85
Day 7	8.00	7.96	7.68	7.62
Day 14	8.00	7.96	7.64	7.35**
Day 21	7.96	7.92	7.64	7.35*
Live birth index	100	99	98	97
Viability index	95	99	98	96
Weaning index	96	99	99	94
F_2 Generation				
Mean Implantation Sites	277	366	372	293
Number born live	252	335	348	277
Number born dead	2	13	10	5
Sex Ratio Day 0 (% ♂)	53	50	48	51
# Deaths Days 0-4 (%)	18 (7.1)	22 (6.6)	17 (4.9)	92 (33.2)
# Deaths Days 4-21 (%)	2 (0.8)	11 (3.3)	5 (1.4)	30 (10.8)
Mean litter size Day 0	12.60	13.96	14.50	12.04
Day 4[b]	11.70	13.61	13.79	8.41**
Day 4[c]	7.10	8.00*	7.96	6.36
Day 7	7.05	7.95*	7.79	6.28
Day 14	7.00	7.86	7.75	6.11
Day 21	7.00	7.86	7.75	6.11
Live birth index	99	97	97	98
Viability index	94	90	95	66**
Weaning index	98	94	97	73**

[a] Data extracted from pages 128-130 and 250-252 of the study report.
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$

2. **Body weight:** Offspring body weights were similar for control, 100 and 280 ppm groups in both generations. Pup weights were reduced at 800 ppm for both the F_1 and F_2 pups at the time of delivery and throughout the lactation period. Selected mean pup body weight data are presented in Table 9.

TABLE 9: Mean Litter and Pup Weights [a]

Lactation Day	0	100	280	800	0	100	280	800
	F_1 Litters (male and female combined)				F_2 Litters (male and female combined)			
0	6.30±0.58	6.19±0.49	6.39±0.53	**5.91±0.46**	6.30±0.77	6.1±0.44	6.15±0.56	**5.96±0.72**
4b	9.57±1.49	9.02±1.19	9.42±1.14	**8.16±1.42**	9.30±1.81	8.64±1.35	8.45±1.27	**7.23±1.93**
4c	9.66±1.47	9.10±1.14	9.58±1.07	**8.30±1.38**	9.43±1.75	8.83±1.34	8.66±1.20	**7.30±1.93**
7	15.5±2.1	14.6±2.0	15.5±2.2	**12.7±2.5**	14.9±2.9	14.4±1.9	13.6±2.6	**11.2±3.0**
14	32.4±2.9	30.5±3.2	32.1±3.1	**26.0±4.3**	30.5±5.3	30.7±2.7	29.4±4.0	**25.1±5.0**
21	49.6±4.1	47.5±5.4	49.1±4.6	**40.1±5.2**	46.7±7.8	47.2±4.9	45.9±6.1	**40.1±7.2**
	F_1 Pups - male				F_2 Pups - male			
0	6.50±0.57	6.41±0.47	6.57±0.65	**6.03±0.48****	6.31±0.83	6.42±0.43	6.49±0.56	**5.97±0.74***
4b	9.76±1.51	9.32±1.23	9.71±1.33	**8.26±1.39****	9.47±1.95	8.85±1.42	8.78±1.23	**7.43±1.92****
4c	9.83±1.52	9.38±1.19	9.87±1.29	**8.37±1.37****	9.66±1.91	9.04±1.39	8.98±1.20	**7.54±1.91****
7	15.9±2.3	15.0±2.2	15.8±2.6	**12.7±2.6****	15.2±3.2	14.6±2.0	13.9±2.7	**11.7±3.1****
21	51.1±4.6	48.8±5.9	50.3±5.6	**39.9±5.8****	48.5±8.3	48.3±5.3	46.8±6.7	**41.2±7.5****
	F_1 Pups - female				F_2 Pups - female			
0	6.11±0.61	6.06±0.48	6.24±0.45	**5.73±0.46****	5.97±0.74	5.98±0.48	6.11±0.54	**5.57±0.62***
4b	9.37±1.51	8.96±1.17	9.20±1.04	**7.89±1.53****	9.06±1.69	8.40±1.35	8.19±1.39	**7.02±2.00****
4c	9.46±1.49	9.05±1.11	9.35±0.95	**8.04±1.45****	9.19±1.64	8.57±1.35	8.29±1.28	**7.22±2.02****
7	15.2±2.0	14.4±1.9	15.0±2.0	**12.5±2.6****	14.7±2.8	13.8±2.0	13.0±2.6	**11.5±3.1****
21	48.7±3.7	47.3±4.1	47.7±3.7	**39.5±4.9****	45.4±7.4	45.8±5.1	44.7±5.6	**40.3±6.7**

[a] Data extracted from pages 130-132 and 252-254 of the study report and pages 8-15 of supplemental pup body weight data.
[b] Before standardization (culling)
[c] After standardization (culling)
* Statistically different from control, $p<0.05$
** Statistically different from control, $p<0.01$ (statistics not available on combined data)

3. **Landmark data for pups:** The mean age to attain vaginal opening was significantly increased for females at 800 ppm. The study author dismissed this observation as incidental because the value was within the range of historical control data from the laboratory. The mean age to attain preputial separation was significantly increased for males at 280 and 800 ppm. The study author dismissed the finding at 280 ppm because it was within the range of historical controls, but concluded that the observation at 800 ppm was suggestive of a treatment-related effect because it was outside the range observed in historical control data from the laboratory as well as historical control data from 3 other laboratories. In the opinion of the reviewer, both the delay in vaginal opening and the delay in age to attain preputial separation observed at 800 ppm are related to treatment with acetamiprid. These findings are consistent with the observed effects on growth and development at that dose. The delay in the age to

attain preputial separation observed at 280 ppm is statistically significant, it is outside the range of in-house historical control animals, and appears to demonstrate a progression in dose-response when compared to concurrent controls and the high-dose group. Based on these considerations, this observation is deemed to be a treatment-related adverse effect. The data are presented in Table 10, below.

Among F_1 pups, eye opening, incisor eruption and pinna unfolding were not affected by treatment. For F_2 pups, eye opening was significantly delayed at 800 ppm and pinna unfolding was delayed, however the difference was not statistically significant. Examination of the incisor eruption data revealed no difference between treated and control animals. Similarly, anogenital measurements for the F_2 pups on lactation day 0 were not affected by treatment.

Table 10: Selected Landmark Data for Pups

Observation (mean age in days)	0 ppm	100 ppm	280 ppm	800 ppm
Vaginal opening (F_1)	31.08±0.91	31.81±1.46	31.80±2.18	33.98±3.62**
Preputial separation (F_1)	41.56±1.83	42.36±1.05	43.32±2.54*	46.48±2.75**
Covance Laboratories historical control: Mean 41.7 days, range 41.14-42.35 days (5 studies) International Life Sciences Institute historical control: Mean 43.6 days, range 41.8-45.9 days (38 studies) Huntingdon Life Sciences historical control: Mean 43.8 days, range 42.8-45.0 days (4 studies) WIL Research Laboratories historical control: Mean 42.9 days, 41.6-44.8 days (8 studies)				
Eye opening (F_2)	14.56±0.69	14.65±0.78	14.35±1.10	15.44±0.92*
Pinna unfolding (F_2)	3.20±0.62	3.48±0.66	3.45±0.97	4.01±1.10

Data obtained from pages 136 and 258 of the study report
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

4. **Offspring postmortem results:**

a) **Organ weights:** There were no organ weight changes observed in 100 or 280 ppm pups that were attributed to treatment with acetamiprid. Mean absolute brain, spleen and thymus weights were significantly decreased for male and female F_1 pups. Changes in relative organ weights at 800 ppm included increased brain-to-body weight in males and females, increased thymus-to-body weight in males, decreased spleen-to-brain weight in males and females and decreased thymus-to-brain weight in females. The data are presented in Table 11, below.

Table 11: Selected organ weight changes in offspring

Observation	0 ppm	100 ppm	280 ppm	800 ppm
		F_1 pups		
Absolute brain weight (g) - males - females	1.52±0.08 1.48±0.09	1.52±0.09 1.48±0.09	1.50±0.08 1.46±0.07	1.39±0.11* 1.36±0.10*
Absolute spleen weight (g) - males - females	0.24±0.05 0.23±0.04	0.24±0.06 0.23±0.05	0.24±0.05 0.24±0.04	0.18±0.04* 0.19±0.05*
Absolute thymus weight (g) - males - females	0.24±0.04 0.26±0.04	0.25±0.06 0.25±0.06	0.26±0.04* 0.26±0.05	0.21±0.05* 0.22±0.06*
Brain-to-body weight (%) - males - females	3.11±0.32 3.15±0.30	3.21±0.42 3.31±0.38	3.12±0.30 3.15±0.24	3.63±0.54* 3.56±0.49*
Thymus-to-body weight (%) - males	0.486±0.071	0.510±0.091	0.536±0.064*	0.529±0.087*
Spleen-to-brain weight ratio - males - females	0.159±0.031 0.158±0.026	0.158±0.034 0.154±0.028	0.157±0.027 0.161±0.024	0.131±0.024* 0.141±0.031*

Thymus-to-brain weight ratio - females	0.173±0.024	0.165±0.040	0.182±0.031	0.158±0.039*
F_2 pups				
Absolute brain weight - males - females	1.48±0.11 1.43±0.09	1.50±0.09 1.43±0.10	1.46±0.12 1.41±0.09	1.38±0.13* 1.35±0.12*
Absolute spleen weight - males - females	0.23±0.05 0.22±0.05	0.23±0.05 0.22±0.05	0.22±0.05 0.22±0.04	0.19±0.05* 0.20±0.05*
Absolute thymus weight - males	0.24±0.04	0.24±0.04	0.25±0.06	0.22±0.06*
Brain-to-body weight - males - females	3.14±0.55 3.25±0.51	3.16±0.37 3.22±0.38	3.22±0.39 3.28±0.41	3.60±0.54* 3.48±0.55*
Thymus-to-body weight - males	0.508±0.055	0.492±0.071	0.536±0.091	0.549±0.104*
Spleen-to-brain weight - males	0.154±0.033	0.157±0.029	0.153±0.030	0.139±0.034*

Data obtained from pages 140-142 and 264-266 of the study report
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

b) Macroscopic examination: There were no treatment-related changes observed at necropsy of offspring from either the F_1 or F_2 pups.

III. DISCUSSION

A. Investigators' conclusions: "In conclusion, the no-observable-effect level (NOEL) in this study for parental and offspring toxicity is 100 ppm (7.5 mg/kg/day) NI-25 in the diet, based on effects on body weight and/or food consumption seen in the 280 ppm males. Dietary exposure of rats to NI-25 up to the highest level (800 ppm) did not result in effects on reproductive performance or fertility. Thus the NOEL for reproductive and fertility parameters is at least 800 ppm (61.8 mg/kg/day)."

B. Reviewer's discussion: A two-generation, one litter per generation reproduction study was conducted using Sprague-Dawley rats, fed test diets containing NI-25 (acetamiprid) at dietary concentrations of 0, 100, 280 or 800 ppm (equal to 0, 6.5, 17.9 or 51.0 mg/kg bw/day for males and 0, 7.6, 21.7 or 60.1 mg/kg bw/day for females) continuously throughout the study period, to 26 rats per sex per group.

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. The study author dismissed these findings as spurious, however, 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 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).**

C. Study deficiencies: None.

DER #5

Acetamiprid: Developmental Toxicity Study in Rats
Nippon Soda. 1997. MRID No. 44651847

Reviewer: Gordon Cockell, Date April 9, 2001

STUDY TYPE: Prenatal Developmental Study - Rat; OPPTS 870.3700; OECD 414.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.46%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Nukui, T. and Y. Fujii (1997) Acetamiprid - Teratogenicity Study in Rats. Toxicology Laboratory, Odawara Research Centre, Nippon Soda Co., Ltd., Odawara, Japan. Laboratory Project ID G-0829, September 29, 1997. MRID 44651847. Unpublished

SPONSOR: Nippon Soda Co., Ltd., Tokyo, Japan

EXECUTIVE SUMMARY: 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.

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

97

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** NI-25

Description:	Pale brown crystal
Lot/Batch #:	NNI-02
Purity:	99.46 % a.i.
Compound Stability:	Stable for 5 years and 1 month in the dark at -20°C
CAS #:	135410-20-7

2. **Vehicle:** 5% gum arabic and 0.01% Tween 80 in water

3. **Test animals:**

Species:	Rat	
Strain:	Crj:CD (SD)	
Age/weight at study initiation:	Approximately 11 weeks of age, males 324-351 g and females 205-243 g	
Source:	Charles River, Japan	
Housing:	Individual, except during the mating period, in suspended stainless steel wire mesh cages	
Diet:	CA-1 diet, from CLEA Japan, Inc., *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21.7±0.3 or 21.8±0.3 °C
	Humidity:	60.0±2.3 or 65.2±2.4 %
	Air changes:	12 times/hr
	Photoperiod:	12 hrs dark / 12 hrs light
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates - Start:** March 3, 1992 **End:** April 1, 1992

2. **Mating:** Sexually mature females were mated with sexually mature males, nightly in a 1:1 ratio. Confirmation of mating was determined by the presence of sperm in the vaginal smear and was designated as day 0 of gestation.

3. **Animal Assignment:** Animals were assigned to groups using a computerized randomization procedure to dose groups as indicated in Table 1.

TABLE 1: Animal Assignment

Dose (mg/kg bw/day)	0	5	16	50
# Females	24	24	24	24

4. **Dose selection rationale:** Doses were selected based on a preliminary investigation that was conducted at doses of 0, 18, 35 or 70 mg/kg bw/day, to 7 mated females per group, on days 6-15 of gestation. Decreased body weight, body weight gain and/or food consumption were reported at 35 and 70 mg/kg bw/day, while no effects were observed at 18 mg/kg bw/day. On the basis of these results, the doses selected for the main study were 0, 5, 16 and 50 mg/kg bw/day.

5. **Dosage preparation and analysis:** Test suspensions were prepared once and used for the entire treatment period. The test suspensions were stored in a refrigerator (approximately 5 °C). A suspension at a nominal concentration of 1240 ppm was prepared and analysed for stability for 4 weeks under refrigeration. Concentration analysis and homogeneity of the test suspensions (top, middle and bottom) were conducted prior to study initiation using HPLC.

Results - **Homogeneity Analysis:** The range of values for the top, middle and bottom of the 5 mg/kg suspension was 98-101% of the target concentration. For the 16 mg/kg suspension, the range of values for the top middle and bottom was also 98-101% of the target concentration. The 50 mg/kg suspension was 95-101% of the target concentration.

Stability Analysis: The results of the stability analysis indicated that after 2 or 4 weeks of storage at 5 °C, the concentration was 99-100% of the week 0 value.

Concentration Analysis: The mean concentration of the test suspensions was 100, 99, and 98% of the target concentration for the 5, 16 and 50 mg/kg dose levels, respectively.

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

6. **Dosage administration:** All doses were administered once daily by oral gavage, on gestation days 6 through 15, in a volume of 2 mL/kg of body weight/day. Dosing was based on the body weight on gestation day 6.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations** - The animals were checked for mortality or clinical signs once daily from the date of first mating to necropsy. Each mated female was given a detailed physical examination twice daily for gestation days 6-15 (once prior to dosing and once 1 hour after dosing). Body weight and food consumption data were recorded on gestation days 0 and 6-21. Dams were sacrificed on day 21 of gestation. The uterus of each animal was excised and weighed prior to the removal of the fetuses, and then opened. The number and location of fetuses and corpora lutea were recorded for each uterine horn. Fetuses were classified as viable (movement in response to touch), nonviable (late death, no movement in response to touch) or resorption (early death, evidence of implantation but no recognizable fetus). When no implantations were grossly apparent, the uterus was stained with 10% ammonium sulfide. When no foci were present, the female was considered sterile. Pre-implantation loss (%) [# corpora lutea - # implantations / # corpora lutea] and post-implantation loss (%) [# implantations - # live fetuses / # implantations] were calculated for each female.

After removal of the uterus, gross post mortem examinations were conducted on each female. Liver, spleen, kidney, adrenal and ovary weights were recorded and organ-to-body weight ratios were calculated using the corrected body weights from gestation day 21 (body weight - gravid uterus weight).

2. **Fetal Evaluations** - Each viable fetus was sexed and given a gross examination for external, palate and optical malformations and variations. Viable fetuses and placentae were weighed individually and the fetuses were tagged for identification. Approximately ½ of the fetuses in each litter were fixed in Bouin's solution and were examined for visceral malformations and variations using Wilson's method

and/or Nishimura's method. The remaining fetuses were fixed in ethanol and stained using Alizarin Red S and Alcian Blue 8GS double staining procedure (McLeod's method). These fetuses were examined for skeletal malformations, variations and ossification defects. The skeletal variation, fourteenth rib, was classified as rudimentary rib or extra rib according to the method of Kimmel *et al* (1973).

D. DATA ANALYSIS

1. Statistical analyses: All statistical analyses were conducted on a per litter basis, comparing the treated group to the concurrent control. Fetal and placental weights were analysed statistically using mean values per litter. The following procedures were employed:

a) Maternal body weight, food consumption, organ weights, fetal and placental weights - Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, one way ANOVA was used, otherwise, the Kruskal-Wallis test was used. If significant differences among the means were apparent, Dunnett's or Scheffe's type tests were used to determine which means were significantly different from the control. Dead or sterile animals were excluded from the calculation of mean maternal body weights, food efficiency and organ weights.

b) Pregnancy rates, number of litters with abnormal fetuses and sex ratios - After setting up 2 x 2 contingency tables, Chi-square test was used to compare the difference between groups. If any one group had a value less than 5, Fisher's exact test was used.

c) Number of corpora lutea, implantations, viable (or nonviable) and abnormal fetuses, and values of pre- and post-implantation losses - Mann-Whitney U test or Fisher's exact test was used to compare the difference between two groups.

In addition, the Cochran-Armitage trend test was performed both on a litter and fetus basis, first on all doses and then after omission of the high-dose, to determine the linear trend in treated groups.

2. Indices: The following indices were calculated from cesarean section records of animals in the study:

Pre-implantation loss: $\frac{\text{Number of corpora lutea - number of implantations}}{\text{Number of corpora lutea}} \times 100$

Post-implantation loss: $\frac{\text{Number of implantations - number of live fetuses}}{\text{Number of implantations}} \times 100$

3. Historical control data: Historical control data were provided to allow comparison with concurrent controls. The data are from 9 studies conducted between 1982 and 1994.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: No mortality or clinical signs of toxicity were observed in any of the treated or control animals.

2. Body Weight: Maternal body weight and body weight gain was significantly decreased at 50 mg/kg bw/day during the treatment period. A slight reduction in body weight gain was apparent at 16 mg/kg

bw/day for the first half of the treatment period (84% of control for days 6-11 of gestation). During the same period, body weight gain among high-dose females was only 18% of controls. During the second half of the treatment period (days 11-15 of gestation), body weight gains was comparable between all groups. Body weight data are summarized in Table 2.

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

Interval	Dose (mg/kg bw/day)			
	Control (N = 23)	5 (N = 23)	16 (N = 24)	50 (N = 23)
Pretreatment: Days 0-6	29.9±7.2	28.6±13.5	30.5±10.0	30.1±8.1
Treatment: Days 6-15	42.1±10.9	43.8±8.0	39.8±9.9	24.8±14.8**
Treatment: Days 6-11 [b]	20.0 (100)	22.6 (113)	16.8 (84)	3.6 (18)
Treatment: Days 11-15 [b]	22.1 (100)	21.2 (96)	23.0 (104)	21.2 (96)
Posttreatment: Days 15-21	84.4±16.1	90.3±16.7	86.0±11.3	95.3±15.1

[a] Data extracted from pages 31-32 of the study report
[b] Data obtained by subtracting group mean body weight on gestation days 6, 11 and 15, hence standard deviations and statistics not available. Body weight gains for these periods are expressed in brackets as % of control
* Significantly different ($p < 0.05$) from the control
** Significantly different ($p < 0.01$) from the control

3. **Food Consumption:** Maternal food consumption was significantly reduced (up to 45%) at 50 mg/kg bw/day, relative to controls, during the first half of the treatment period (gestation days 6-11). A slight increase in maternal food consumption relative to controls was observed at 50 mg/kg bw/day during the post-treatment period. There were no other remarkable differences in maternal food consumption between treated and control groups.

4. **Maternal Organ Weights:** Absolute liver weight, liver-to-body weight and left kidney-to-body weight were significantly increased at 50 mg/kg bw/day. The study author only considered the liver weight changes to be related to treatment with acetamiprid. The reviewer concurs that the slight increase in the left kidney-to-body weight ratio is an incidental finding. All other organ weight data was comparable between treated and control groups.

5. **Gross Pathology:** There were no treatment-related macroscopic changes observed in any animals at necropsy. Only two gross lesions were apparent, each present in one animal, however neither was deemed to be related to treatment. Splenodiaphragmatic adhesion was observed in one mid-dose female, and splenic hypertrophy was observed in one high-dose female.

6. **Cesarean Section Data:** No treatment-related changes were observed. No premature delivery or abortion occurred and the pregnancy rates were comparable between controls and treated animals. The number of resorbed fetuses (early death) and post-implantation loss were significantly increased at 16 mg/kg bw/day, however, neither of these parameters was affected at the high dose, therefore the differences noted at 16 mg/kg bw/day were considered incidental. There were no treatment-related changes in the number of fetuses per dam, fetal sex ratios, or fetal weights. Cesarean section observations are summarized in Table 3.

TABLE 3 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	5	16	50
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant	23	23	24	23
Pregnancy Rate (%)	96	96	100	96
# Nonpregnant	1	1	0	1
Maternal Wastage				
# Died	0	0	0	0
# Died Pregnant	0	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	476 20.7±3.3	447 19.4±2.7	500 20.8±4.1	462 20.1±3.5
Total # Implantations (Implantations/Dam)	357 15.5±2.4	356 15.5±2.4	380 15.8±1.8	342 14.9±3.6
Total # Litters	23	23	24	23
Total # Live Fetuses (Live Fetuses/Dam)	345 15.0±2.4	340 14.8±2.5	355 14.8±1.9	324 14.1±3.8
Total # Resorptions (%)	12 (3.4)	16 (4.5)	25 (6.6)*	18 (5.3)
Early	12 (3.4)	15 (4.2)	24 (6.3)*	17 (5.0)
Late	0 (0.0)	1 (0.3)	1 (0.3)	1 (0.3)
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)				
Males	5.277±0.289	5.363±0.336	5.121±0.317	5.211±0.282
Females	4.954±0.388	5.098±0.354	4.946±0.290	4.930±0.336
Sex Ratio (% Male)	48.4	50.0	45.1	53.7
Preimplantation Loss (%)	23.6±15.4	19.0±15.8	21.8±13.5	26.2±16.7
Postimplantation Loss (%)	3.4±3.4	4.4±5.8	6.7±5.5*	7.3±14.1

[a] Data extracted from pages 36 and 63-66 of the study report.
* Significantly different ($p < 0.05$) from the control.
** Significantly different ($p < 0.01$) from the control.

B. DEVELOPMENTAL TOXICITY

1. **External Examination:** There were no external malformations related to treatment with acetamiprid. The only external malformation observed was in one fetus in the control group, which had a short tail. The incidence of two external variations was slightly increased in the high dose group. The incidence of placental hemorrhage was 0, 0, 0, and 3 fetuses (2 litters) for the control, 5, 16 and 50

mg/kg bw/day dose groups, respectively. The incidence of subcutaneous hemorrhage was 2 fetuses (2 litters), 1 fetus (1 litter), 1 fetus (1 litter) and 5 fetuses (4 litters) for the control, 5, 16 and 50 mg/kg bw/day dose groups, respectively. The study author did not consider these findings to be related to treatment with acetamiprid. None of the differences were statistically significant, however there was a positive trend using the Cochran-Armitage test for the incidence of placental hemorrhage. The reviewer concurs with the study author's interpretation that these findings are likely incidental and not related to treatment with acetamiprid.

TABLE 4a. External Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	5	16	50
#Fetuses (litters) examined	345 (23)	340 (23)	355 (24)	324 (23)
Short tail	1 (1)[b]	0(0)	0(0)	0(0)
Placental hemorrhage	0 (0)	0 (0)	0 (0)	3 (2)[c]
Subcutaneous hemorrhage	2 (1)	1 (1)	1 (1)	5 (4)

[a] Data extracted from page 37 of the study report.
[b] Fetal (litter) incidence
[c] $p < 0.05$, Cochran-Armitage trend test

2. Visceral Examination: There were no visceral malformations observed in the study. The overall incidence of fetuses with visceral variations was 22, 15, 24 and 8 for the control, 5, 16 and 50 mg/kg bw/day dose groups, respectively. The variations that occurred most frequently, at similar rates among treated and control animals included dilatation of the renal pelvis and thymic remnants in the neck. None of these observations were deemed to be related to treatment with acetamiprid. The reviewer concurs with this conclusion.

TABLE 4b. Visceral Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	5	16	50
#Fetuses (litters) examined	174 (23)	175 (23)	180 (24)	162 (23)
Dilatation of the renal pelvis	13 (8)[b]	8 (7)	19 (16)	10 (7)
Thymic remnant in the neck	8 (7)	7 (7)	5(4)	3 (3)
Total fetuses with soft tissue variations	22 (13)	15 (12)	24 (17)	13 (9)

[a] Data extracted from pages 37 and 67-255 of the study report.
[b] Fetal (litter) incidence

3. Skeletal Examination: The data provided on ossification processes showed no evidence of differences between control and treated animals. The study author reported that treatment with acetamiprid did not result in any skeletal malformations, variations or retarded ossification in fetuses. The incidence of fetuses (litters) with skeletal malformations was 3 (3), 0 (0), 2 (2) and 1 (1) for the control, 5, 16 and 50 mg/kg bw/day dose groups, respectively. The reviewer concurs that there were no treatment-related skeletal malformations nor any changes in ossification processes observed in the study. However, the incidence of the skeletal variation, "shortening of the rib", was increased among high-dose fetuses (see Table 4c), both on a fetal and litter basis. The study author dismissed this as not related to treatment based on the lack of a dose response at the two lower doses. The in-house

historical control data for this observation, from a total of 9 studies, indicated a mean incidence of 0.5% with a range of 0.0-1.3%. While it is true that there is no apparent trend in the lower two doses, the significance of the observation at the high dose cannot be dismissed, particularly when considered in relation to the historical control range for this finding.

TABLE 4c. Skeletal Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	5	16	50
#Fetuses (litters) examined	171 (23)	165 (23)	175 (24)	162 (22)[b]
Malformations (Fetal (litter) incidence)				
Defect of postcervical vertebrae	1 (1)	0 (0)	0 (0)	0 (0)
Partial hypertrophy of ribs	1 (1)	0 (0)	1 (1)	1 (1)
Fusion of the ribs	1 (1)	0 (0)	0 (0)	0 (0)
Bifurcation of the rib	1 (1)	0 (0)	0 (0)	0 (0)
Fusion of the sternebrae	0 (0)	0 (0)	1 (1)	0 (0)
Total fetuses (litters) with malformations	3 (3)	0 (0)	2 (2)	1 (1)
Variations (Incidence, N (%))				
Splitting of cervical vertebral body	10 (5.8)	10 (6.1)	10 (5.7)	11 (6.8)
Asymmetry of the sternebrae	11 (6.4)	16 (9.7)	21 (12.0)	15 (9.3)
Shortening of the rib	1 (0.6)	6 (3.6)	1 (0.6)	15 (9.3)***
Total fetuses with skeletal variations	37 (21.3)	47 (28.5)	44 (25.1)	47 (29.0)
Ossification (Mean±SD)				
No. of cervical vertebral bodies	4.9±1.3	4.6±1.2	4.9±1.3	4.3±1.3
No. of sternebrae	6.0±0.1	6.0±0.0	6.0±0.0	6.0±0.1
No. of post-lumbar vertebrae	10.8±0.7	10.8±0.6	10.6±0.6	10.3±0.9
No. of distal phalanges - Fore - Hind	8.3±1.8 8.5±1.2	8.6±1.1 9.0±0.9	8.5±1.6 8.8±1.3	8.0±2.5 8.3±1.6
No. of proximal phalanges - Fore - Hind	4.5±1.6 1.3±1.2	5.1±1.6 1.8±1.6	4.3±1.6 1.1±1.1	4.8±2.3 1.3±1.5
No. of metacarpals	8.0±0.1	8.0±0.0	8.0±0.1	7.9±0.2
No. of metatarsals	9.2±0.6	9.3±0.5	9.0±0.6	8.9±0.8

[a] Data extracted from page 38 of the study report.
[b] Female #1303 delivered one viable fetus; skeletal examination was not performed
*** Significantly different ($p < 0.001$) from the control.

III. DISCUSSION

A. Investigators' conclusions: "Based on the decreases of food consumption and growth depression during the treatment period, and the increases of liver weight at scheduled sacrifice noted in maternal rats for the 50 mg/kg/day group, the maximum no observable effect level of NI-25 in a 5% arabic gum and 0.01% Tween 80 aqueous vehicle, under the condition of the study, is considered to be 16 mg/kg/day.

The treatment of NI-25 did not produce fetal toxic or teratogenic response, when treated to pregnant rats by gastric intubation at dose levels of 5, 16 and 50 mg/kg/day for a period of 10 days (gestation days 6-15)."

B. Reviewer's discussion: Groups of 24 pregnant Crj:CD(SD) rats were treated with acetamiprid by oral gavage on gestation days 6-15 at dose levels of 0, 5, 16 or 50 mg/kg bw/day. There was no mortality, nor were there any clinical signs of toxicity recorded in the study. There were no abortions or premature deliveries. Treatment with acetamiprid did not affect pregnancy rate, implantations, resorptions, number of corpora lutea or uterine weights. There were no treatment-related macroscopic pathology findings, nor were there any treatment-related changes in fetal sex ratio, number of fetuses, fetal weights, or fetal external and visceral examinations. Among the dams, decreased body weight, body weight gain and food consumption were observed at 50 mg/kg bw/day. Absolute and relative liver weights were also increased at that dose. In the fetal skeletal examinations, a significant increase in the incidence of shortened 13th rib was observed at 50 mg/kg bw/day, however this was dismissed by the study author due to a lack of a dose-related trend at the two lower doses. The reviewer considers this finding to be related to treatment.

The NOAEL for maternal toxicity is 16 mg/kg bw/day, based on the observed reductions in body weight, body weight gain, food consumption and the increased liver weights observed at 50 mg/kg bw/day. The maternal LOAEL is 50 mg/kg bw/day.

The NOAEL for developmental toxicity is 16 mg/kg bw/day, based on the observed increase in the incidence of shortening of the 13th rib at 50 mg/kg bw/day. The developmental LOAEL is 50 mg/kg bw/day.

There was no evidence of any teratogenic effects due to treatment with acetamiprid.

1. Maternal toxicity: Maternal body weight, body weight gain and food consumption were reduced at 50 mg/kg bw/day. Mean absolute and relative liver weights were increased at 50 mg/kg bw/day. There were no other treatment-related observations among maternal animals in the study.

2. Developmental toxicity: Developmental toxicity was apparent at 50 mg/kg bw/day, notably an observed increase in the incidence of shortening of the 13th rib.

a. Deaths/Resorptions: There were no treatment-related deaths or resorptions in the study.

b. Altered Growth: Treatment with acetamiprid did not affect fetal body weights.

c. Developmental Variations: The incidence of the skeletal variation, shortening of the 13th rib, was increased at 50 mg/kg bw/day, both on a fetal and litter basis.

d. Malformations: There were no treatment-related malformations observed in this study.

C. **Study deficiencies:** None.

Acetamiprid: Developmental Toxicity Study in Rabbits
Nippon Soda Company. 1997. MRID No. 44651848

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PMRA ARLA

Reviewer: Gordon Cockell, Date May 11, 2001

STUDY TYPE: Prenatal Developmental Study - Rabbit; OPPTS 870.3700; OECD 414.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.46%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Nukui, T. and Y. Fujii (1997) Acetamiprid - Teratogenicity Study in Rabbits. Toxicology Laboratory, Odawara Research Centre, Nippon Soda Co., Ltd., Odawara, Japan. Laboratory Project ID G-0830, September 29, 1997. MRID 44651848. Unpublished

SPONSOR: Nippon Soda Co., Ltd., Tokyo, Japan

EXECUTIVE SUMMARY: 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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** NI-25

Description:	Pale brown crystal
Lot/Batch #:	NNI-02
Purity:	99.46 % a.i.
Compound Stability:	Stable for 5 years and 1 month in the dark at -20°C
CAS #:	135410-20-7

2. **Vehicle:** 5% gum arabic and 0.01% Tween 80 in water

3. **Test animals:**

Species:	Rabbit	
Strain:	Kbs:NZW	
Age/weight at study initiation:	Approximately 5 months of age, males 3547.9-4513.7 g and females 3193.8-4298.3 g	
Source:	Kitayama Labes Co., Ltd., Nagano, Japan	
Housing:	Individual, except during the mating period, in suspended stainless steel wire mesh cages	
Diet:	RC-4 diet, from Oriental Yeast Co., Ltd., Japan, *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	**Temperature:**	20.7±0.5 °C
	Humidity:	64.4±2.9 %
	Air changes:	12 times/hr
	Photoperiod:	12 hrs dark / 12 hrs light
Acclimation period:	6 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates - Start:** February 4, 1993 **End:** March 16, 1993

2. **Mating:** One randomly selected male was placed in a cage with one female and cageside observation was used to detect copulation. When copulation had been observed twice, the female was considered to have successfully mated. The day on which the second occurrence of copulation was observed was designated as day 0 of gestation.

3. **Animal Assignment:** Animals were assigned to groups using a computerized randomization procedure to dose groups as indicated in Table 1.

TABLE 1: Animal Assignment

Dose (mg/kg bw/day)	0	7.5	15	30
# Females	17	17	17	17

4. **Dose selection rationale:** Doses were selected based on a preliminary investigation that was conducted at doses of 0, 5, 13, 30 or 75 mg/kg bw/day, to 4 mated females per group, on days 6-18 of gestation. At 75 mg/kg bw/day, all animals died by gestation day 14. Decreased body weight, body weight gain and/or food consumption was observed at 30 mg/kg bw/day, and one animal at this dose

aborted on gestation day 26. A slight decrease in body weight and food consumption was observed at 13 mg/kg bw/day. On the basis of these results, the doses selected for the main study were 0, 7.5, 15 and 30 mg/kg bw/day.

5. **Dosage preparation and analysis:** Test suspensions were prepared in two batches and used for the entire treatment period. The test suspensions were stored in a refrigerator (approximately 5 °C). A suspension at a nominal concentration of 1240 ppm was prepared and analysed for stability for 4 weeks under refrigeration. Concentration analysis and homogeneity of the test suspensions (top, middle and bottom) were conducted prior to study initiation using HPLC.

Results - **Homogeneity Analysis:** The range of values for the top, middle and bottom of the 7.5 mg/kg suspension was 92-97% of the target concentration. For the 15 mg/kg suspension, the range of values for the top middle and bottom was also 93-105% of the target concentration. The 30 mg/kg suspension was 95-101% of the target concentration.

Stability Analysis: The results of the stability analysis indicated that after 2 or 4 weeks of storage at 5 °C, the concentration was 99-100% of the week 0 value.

Concentration Analysis: The mean concentration of the first batch of the test suspensions was 95, 98, and 99% of the target concentration for the 7.5, 15 and 30 mg/kg dose levels, respectively. The mean concentration of the second batch of the test suspensions was 94, 95, and 96% of the target concentration for the 7.5, 15 and 30 mg/kg dose levels, respectively.

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

6. **Dosage administration:** All doses were administered once daily by oral gavage, on gestation days 6 through 18, in a volume of 4 mL/kg of body weight/day. Dosing was based on the body weight on gestation day 6.

C. OBSERVATIONS

1. **Maternal Observations and Evaluations** - The animals were checked for mortality or clinical signs once daily from the date of first mating to necropsy. Each mated female was given a detailed physical examination daily for gestation days 6-28 (approximately 1 hour after dosing). Body weight and food consumption data were recorded on gestation days 0 and 6, 7, 8, 10, 12, 14, 16, 18, 19, 20, 22, 24 and 28. Dams were sacrificed on day 28 of gestation. The uterus of each animal was excised and weighed prior to the removal of the fetuses, and then opened. The number and location of fetuses and corpora lutea were recorded for each uterine horn. Fetuses were classified as viable (movement in response to touch), dead (body and limbs evident, no degeneration of tissue), macerated (limbs evident and show white malacia (autolysis)), resorption (placenta and embryo being resorbed seen), placenta (only placenta is seen) or implantation site (only implantation site is seen). Pre-implantation loss (%) [# corpora lutea - # implantations / # corpora lutea] and post-implantation loss (%) [# implantations - # live fetuses / # implantations] were calculated for each female.

After removal of the uterus, gross post mortem examinations were conducted on each female. Liver, spleen, kidney, adrenal and ovary weights were recorded and organ-to-body weight ratios were calculated using the corrected body weights from gestation day 28 (body weight - gravid uterus weight).

2. **Fetal Evaluations** - Each viable fetus was sexed and given a gross examination for external, palate and optical malformations and variations. Viable fetuses and placentae were weighed individually and the fetuses were tagged for identification. If the fetal body weight was less than 60% of the control mean, the fetus was considered underdeveloped. The fetuses were sacrificed by chloroform inhalation and retained in ethanol after intrathoracic and intraperitoneal injection of ethanol. After fixation, the fetuses were subject to examination for visceral malformations and variations using Tanimura's method. Each animal was examined for cervical, thoracic, abdominal pelvic and cranial organs. The viscera and brain were excised and observed. After removal of the viscera, the skin was removed and the skeleton was fixed in acetone and stained using Alizarin Red S. The fetuses were examined for skeletal malformations, variations and ossification defects.

D. DATA ANALYSIS

1. **Statistical analyses:** All statistical analyses were conducted on a per fetus and a per litter basis, comparing the treated group to the concurrent control. Fetal and placental weights were analysed statistically using mean values per litter. The following procedures were employed:

a) Maternal body weight, food consumption, organ weights, fetal and placental weights, number of implantations, corpora lutea, viable fetuses and fetal skeletons - Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, one way ANOVA was used, otherwise, the Kruskal-Wallis test was used. If significant differences among the means were apparent, Dunnett's or Scheffe's type tests were used to determine which means were significantly different from the control. Dead or sterile animals were excluded from the calculation of mean maternal body weights, food efficiency and organ weights.

b) Fetal mortality, incidences of litters containing fetuses with malformations/variations, fetal sex ratios and incidences of fetuses with malformations/variations - After setting up 2 x 2 contingency tables, Chi-square test was used to compare the difference between groups. If any one group had fewer than 5 data points, Fisher's exact test was used.

c) Extent of fetal ossification (cranial bone) - Kruskal-Wallis test was used.

d) Pre- and post-implantation losses - Mann-Whitney U test was used to compare the difference between two groups.

In addition, the Cochran-Armitage trend test was performed to determine the linear trend in treated groups.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

Pre-implantation loss: $\frac{\text{Number of corpora lutea - number of implantations}}{\text{Number of corpora lutea}} \times 100$

Post-implantation loss: $\frac{\text{Number of implantations - number of live fetuses}}{\text{Number of implantations}} \times 100$

3. **Historical control data:** Historical control data were provided to allow comparison with concurrent controls. The data are from 5 studies conducted between 1983 and 1993.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: There were no treatment-related mortalities in the study. The observed mortalities were attributed to "inadequate treatment or restraint", and the incidence was 0, 3, 2 and 1 animal in the control, 7.5, 15 and 30 mg/kg bw/day dose groups, respectively. The corresponding pregnancy rates for these groups were 70.6%, 88.2%, 82.4% and 82.4%. There were no treatment-related clinical signs of toxicity in the study. One control animal and one low-dose animal showed prone position and abnormal gait (lumbar paralysis).

2. Body Weight: The study author reported treatment-related depression of maternal body weight. A group mean loss of 7.3 grams occurred at 30 mg/kg bw/day over the entire treatment period (study days 6-19), compared to a gain of 35.2 grams in the control group over the same interval. The data for the interval of days 6-19 were highly variable in all of the groups including controls, such that an effect of treatment could not be clearly demonstrated. However, examination of the body weight data from the initial part of the treatment period (study days 6-10) shows a treatment-related increase in body weight loss among high-dose animals. Body weight loss over the first four days of treatment was noted among controls, mid- and high-dose animals, and this observation was more pronounced at 30 mg/kg bw/day. Body weight data are summarized in Table 2.

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

Interval	Dose (mg/kg bw/day)			
	Control (N = 12)	7.5 (N = 12)	15 (N = 12)	30 (N = 13)
Pretreatment: Days 0-6	84.8±69.2	77.3±72.8	121.6±40.4	73.6±124.6
Treatment: Days 6-10	-16.1±43.2	2.9±43.8	-36.5±67.3	-100.9±77.4
Treatment: Days 6-19	35.2±134.0	40.3±168.2	66.3±102.1	-7.3±203.9
Posttreatment: Days 19-28	174.9±168.1	71.1±249.4	0.2±247.3	166.5±170.1
Total: Days 0-28	294.9±173.1	188.7±309.8	188.1±261.1	232.8±335.3

[a] Data extracted from page 40 of the study report

3. Food Consumption: A significant reduction in food consumption was observed among high-dose animals on study days 6-8. This is consistent with the increased body weight loss at that dose over the first four days of the treatment period. Food consumption for the remainder of the treatment period and through to study termination was similar between controls and all of the treated groups of animals.

4. Maternal Organ Weights: There were no treatment-related changes in maternal organ weights.

5. Gross Pathology: There were no macroscopic observations that were attributed to treatment with acetamiprid. Among animals that died "due to inadequate treatment or restraint", the following lesions were observed: perforation of the lung, dark red or red patches in the lung, red fluid on the tracheal mucosa and/or red fluid in the thoracic cavity. Among animals that survived to study termination, pale brown or black patches in the lung, red fluid on the tracheal mucosa and/or prominent liver lobules were noted occasionally in treated and control animals.

///

6. Cesarean Section Data: There were no treatment-related changes observed in any of the cesarean section observations. Cesarean section observations are summarized in Table 3.

TABLE 3 Cesarean Section Observations [a]

Observation	Dose (mg/kg bw/day)			
	0	7.5	15	30
# Animals Assigned (Mated)	17	17	17	17
# Animals Pregnant	12	15	14	14
Pregnancy Rate (%)	70.6	88.2	82.4	82.4
# Nonpregnant	5	2	3	3
Maternal Wastage				
# Died	0	3	2	1
# Died Pregnant	0	3	2	1
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	0	0
# Premature Delivery	0	0	0	0
Total # Corpora Lutea Corpora Lutea/Dam	132 11.0±2.2	141 11.8±2.4	134 11.2±1.9	121 9.3±2.5
Total # Implantations (Implantations/Dam)	105 8.8±3.0	109 9.1±3.4	110 9.2±2.7	94 7.2±3.3
Total # Litters	12	12	12	13
Total # Live Fetuses (Live Fetuses/Dam)	93 7.8±3.0	99 8.3±2.7	94 7.8±2.7	86 6.6±3.2
Total # Resorptions (%)	12 (11.4)	10 (9.2)	16 (14.5)	8 (8.5)
Early	10	3	5	3
Late	2	7	11	5
Litters with Total Resorptions	0	0	0	0
Mean Fetal Weight (g)				
Males	42.91±5.74	41.59±7.53	39.38±6.62	42.13±8.76
Females	40.11±8.60	40.18±9.38	37.01±7.00	40.51±9.28
Sex Ratio (% Male)	52.7	49.5	50.0	53.5
Preimplantation Loss (%)	20.8±22.0	23.2±21.8	19.2±19.7	26.1±24.6
Postimplantation Loss (%)	11.4±15.6	8.6±12.7	13.5±19.0	7.3±10.9

[a] Data extracted from page 45 of the study report.

B. DEVELOPMENTAL TOXICITY

1. **External Examination:** The study author reported that there were no external malformations or variations related to treatment with acetamiprid. Two external malformations were observed in one fetus from the 30 mg/kg bw/day group, open eyelid and manus varus (clubhand). There were no external variations observed in any of the control or treated groups. The results of the external examinations are reported in Table 4a.

TABLE 4a. External Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	7.5	15	30
#Fetuses (litters) examined	93 (12)	99 (12)	94 (12)	86 (13)
Open eyelid	0 (0)[b]	0(0)	0(0)	1(1)
Manus varus (clubhand)	0 (0)	0 (0)	0 (0)	1 (1)

[a] Data extracted from page 48 of the study report.
[b] Fetal (litter) incidence

2. **Visceral Examination:** There were no treatment-related visceral malformations or variations. One fetus in each of the control, low and high-dose groups had one visceral malformation. Abnormal origin of the right subclavian artery was observed in one fetus from the control group, bifid apex of the heart was observed in one fetus from the low-dose group and microphthalmia was observed in one fetus from the high-dose group. The results of the visceral examinations are reported in Table 4b, below.

TABLE 4b. Visceral Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	7.5	15	30
#Fetuses (litters) examined	93 (12)	99 (12)	94 (12)	86 (13)
Malformations				
Microphthalmia	0 (0)[b]	0 (0)	0 (0)	1 (1)
Abnormal origin of right subclavian artery	1 (1)	0 (0)	0 (0)	0 (0)
Bifid apex of heart	0 (0)	1 (1)	0 (0)	0 (0)
Total fetuses with visceral malformations	1 (1)	1 (1)	0 (0)	1 (1)
Variations				
Dilatation of the renal pelvis	3 (3)	3 (3)	5 (4)	1 (1)
Thymic remnant in the neck	0 (0)	0 (0)	0 (0)	1 (1)
Total fetuses with soft tissue variations	3 (3)	3 (3)	5 (4)	2 (2)

[a] Data extracted from page 37 of the study report.
[b] Fetal (litter) incidence

3. **Skeletal Examination:** There were no treatment-related skeletal malformations, variations nor delays in ossification observed in the study. The results of the skeletal examinations are presented in Table 4c, below.

TABLE 4c. Skeletal Examinations [a]

Observations	Dose (mg/kg bw/day)			
	0	7.5	15	30
#Fetuses (litters) examined	93 (12)	99 (12)	94 (12)	86 (13)
Malformations (Fetal (litter) incidence)				
Vertebral malformation with or without associated rib malformation [b]	1 (1)	1 (1)	2 (2)	2 (2)
Fusion of the sternebrae	3 (3)	3 (3)	1 (1)	2 (2)
Total fetuses (litters) with malformations	4 (4)	4 (3)	3 (3)	4 (4)
Variations (Fetal (litter) incidence)				
Cervical rib	1 (1)	0 (0)	0 (0)	0 (0)
Splitting of sternebrae	3 (2)	0 (0)	2 (2)	1 (1)
Asymmetry of the sternebrae	1 (1)	0 (0)	0 (0)	0 (0)
Bilobed shape of sternebrae	1 (1)	1 (1)	0 (0)	1 (1)
Shortening of the 13th rib	3 (3)	16 (7)**	6 (3)	4 (4)
Nodulated rib	0 (0)	1 (1)	0 (0)	0 (0)
13th rib	62 (11)	63 (12)	42 (11)**	60 (13)
Floating rib	14 (7)	12 (7)	10 (7)	4 (4)*
Total fetuses with skeletal variations	66 (11)	64 (12)	43 (11)	60 (13)
Ossification (Mean±SD)				
No. of cervical vertebral bodies	7.00±0.00	7.00±0.00	7.00±0.00	7.00±0.00
No. of sternebrae	5.85±0.15	5.85±0.18	5.95±0.08	5.86±0.19
No. of sacral and caudal vertebrae	19.77±0.52	19.51±0.60	19.42±0.60	19.91±0.58
No. of distal phalanges - Fore - Hind	9.98±0.06 8.00±0.00	10.00±0.00 8.00±0.00	10.00±0.00 8.00±0.00	10.00±0.00 8.00±0.00
No. of proximal phalanges - Fore - Hind	9.96±0.10 8.00±0.00	10.00±0.00 8.00±0.00	10.00±0.00 8.00±0.00	10.00±0.00 8.00±0.00
No. of metacarpals	9.88±0.23	9.84±0.32	9.98±0.06	9.86±0.33
No. of metatarsals	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00

[a] Data extracted from pages 52-56 of the study report.
[b] This group of observations includes absence or fusion of thoracic vertebral arch, absence of thoracic vertebral body, absence of lumbar vertebral arch, with or without fusion or bifurcation of the ribs.
* Significantly different ($p < 0.05$) from the control
** Significantly different ($p < 0.01$) from the control

III. DISCUSSION

A. Investigators' conclusions: "The treatment of NI-25 (suspended in 5% arabic gum and 0.01% Tween 80 in water) did not produce fetal toxic and teratogenic response when treated orally to pregnant rabbits by gastric intubation at dose levels of 7.5, 15 and 30 mg/kg/day for a period of 13 days from gestation day 6 to 18. The maximum no observable effect level of NI-25 in fetuses is considered to be 30 mg/kg/day or over.

"Based on the decreases of food consumption and growth depressions during the treatment period noted in maternal rabbits for the 30 mg/kg/day group, the maximum no observable effect level of NI-25, under the condition of this study, is considered to be 15 mg/kg/day."

B. Reviewer's discussion: Acetamiprid was administered to 17 New Zealand White rabbits per group via oral gavage at doses of 0, 7.5, 15 or 30 mg/kg bw/day on 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. No signs of developmental toxicity were observed in this study. Treatment with acetamiprid did not affect fetal growth or development and there were no treatment-related developmental variations or malformations reported in the study.

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.

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.

1. Maternal toxicity: A significant reduction in maternal food consumption, with a concomitant slight loss of maternal body weight, was observed during the first few days of dosing. There were no other treatment-related changes observed in any of the parameters investigated in this study.

2. Developmental toxicity:
a. Deaths/Resorptions: Treatment with acetamiprid did not affect the incidence of fetal deaths or resorptions.
b. Altered Growth: Treatment with acetamiprid did not affect fetal growth.
c. Developmental Variations: There were no treatment-related developmental variations in this study.
d. Malformations: There were no treatment-related malformations observed in this study.

C. Study deficiencies: None.

115

Acetamiprid: 13-Week Feeding Study in Rats
Nippon Soda. 1997. MRID No. 44651843

DATA EVALUATION RECORD

ACETAMIPRID
(31-1359)

STUDY TYPE: 90-DAY ORAL TOXICITY - RAT [OPPTS 870.3100 (§82-1a)]
MRID 44651843

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Task Order No. 01-78A

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Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral (Dietary) Toxicity Study - Rat [OPPTS Number:870.3100 (§82-1a)]

DP BARCODE: D264156 SUBMISSION CODE: S575947
P.C. CODE: 099050 TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): 31-1359 (Acetamiprid) (>99% purity)

SYNONYMS: Code No.: 31-1359; (E)-N^1-[(6-chloro-3-pyridyl)methyl)]-N^2-cyano-N^1-methyl-acetamidine

CITATION: Nukui, T., and Ikeyama, S. (1997) Acetamiprid - Thirteen-week dietary subchronic toxicity study in rats. Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., 345 Takada, Odawara, Kanagawa, Japan 250-02. Laboratory Project No. G-0768, September 29, 1997. MRID 44651843. Unpublished.

SPONSOR: Nippon Soda Co., Ltd., 2-2-1 Ohtemachi, Chiyodaku, Tokyo, Japan 100

EXECUTIVE SUMMARY: 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.

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

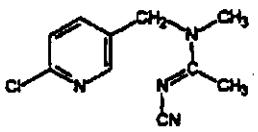
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Description: white solid
Lot No.: 31-0223-HY (Tox-447)
Purity: >99%
Stability of compound: Stable for 6 years in the dark at -20°C

Structure:



2. Vehicle and/or positive control

The test material was administered in the diet (MF powdered basal diet); no positive control was used in this study.

3. Test animals

Species: rat
Strain: Crj:CD (Sprague-Dawley)
Age and weight at study initiation: approximately 6 weeks old; males:157.5-190.5 g; females: 137.3-161.1 g
Source: Charles River Japan Inc. (Kanagawa)
Housing: individually housed in suspended stainless steel, wire-mesh cages
Diet: powdered diet (MF, Oriental Yeast Co., Ltd., Tokyo) was available *ad libitum*
Water: tap water was available *ad libitum*
Environmental conditions:
Temperature: 21.7±0.2°C
Humidity: 59.4±2.5%
Air changes: 15 fresh air changes per hour
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 1 week

B. STUDY DESIGN

1. In life dates

Start: February 12, 1991; end: May 17, 1991

2. Animal assignment

Animals were randomly assigned to the test groups in Table 1 based on a computer-assisted randomization procedure. Body weight means of each group were comparable.

TABLE 1: Study design					
Test Group	Conc. in Diet (ppm)	Mean dose to animal (mg/kg/day)		Number of animals	
		male	female	male	female
Control	0	0.0	0.0	10	10
Low	50	3.1	3.7	10	10
Low-mid	100	6.0	7.2	10	10
Mid-	200	12.4	14.6	10	10
High-mid	800	50.8	56.0	10	10
High-	1600	99.9	117.1	10	10

Data taken from p. 26 and Text Table II, p. 32, MRID 44651843.

3. Dose selection rationale

Dose selection was based on the results of a preliminary, two-week dietary toxicity study in rats. Groups of 4 male and 4 female F344 rats 6 weeks of age were administered 31-1359 in the diet at concentrations of 0, 100, 400, or 2000 ppm for 14 days. A summary of the study results was supplied in a one page summary in Annex 4 (p. 373 of MRID 44651843). Results were stated as an increase or decrease in the endpoint noted; actual values were not provided. No effects were observed at 100 ppm. At 400 ppm, body and spleen weights were decreased in males and free cholesterol was increased in males and females. Numerous effects were observed in animals fed 2000 ppm, including differences in hematology (increased erythrocyte count, hematocrit, hemoglobin concentration, and erythrocyte indices in females and/or males; decreased platelet count in females), urinalysis (increased specific gravity and decreased sodium excretion in females; decreased water consumption, urine volume, and potassium excretion in males and females), clinical biochemistry (increased total and free cholesterol and total protein in males and females; increased GTP and cholinesterase in males), absolute organ weights (increased liver weights and decreased thymus and spleen weights in males and females; decreased lung and kidney weights in males; decreased ovary weights in females), relative organ weights (increased liver and adrenal weights and decreased spleen weights in males and females; increased kidney weights in males), and microscopic lesions (centrilobular hepatocellular hypertrophy in males and females; fatty degeneration in males). Based on these results, dose levels of 0, 50, 100, 200, 800, or 1600 ppm were chosen for the subchronic oral toxicity study.

4. Test material preparation and analysis

Diet was prepared three times during the study by mixing appropriate amounts of test substance with a small amount of basal diet (MF), grinding to a fine powder, and mixing the premix with the remaining amount of basal diet. The test diet was stored at -16 to -21°C. For each diet preparation, a sample was taken from the top, middle, and bottom of the diet mixture from each dose level for concentration and homogeneity analyses. Only one sample was analyzed for the control level. A 50 ppm test diet was prepared and analyzed for stability following 7 days at room temperature and for up to 5 weeks in the freezer (-18 to -20°C).

Results –
Homogeneity Analysis: The coefficient of variation for the 50, 100, 200, 800, or 1600 ppm diets was 7.6, 3.9, 3.8, 5.7, and 4.4%, respectively, indicating that the test substance was homogeneously mixed in the diets.

Stability Analysis: The stability analysis revealed that the test material was stable in the test diet when stored at room temperature for one week (concentration at one week was 95% of the mean concentration measured at day 0) and when stored frozen for 2 weeks (101% of day 0 mean) and 5 weeks (103% of day 0 mean).

Concentration Analysis: The ranges of mean measured concentrations for the 50, 100, 200, 800, or 1600 ppm test diets were: 46.1-54.1 ppm (92-108%), 100-107 ppm (100-107%), 202-216 ppm (101-108%), 782-870 ppm (98-109%) and 1589-1723 ppm (99-108%), respectively.

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

5. Statistics

Mortality, clinical observations, and ophthalmological and macroscopic observations were analyzed using the Chi-square test. The Mann-Whitney U test was used for semi-quantitative urinalysis values and microscopic observations, and the multiple comparison procedure was used to compare body weights, food consumption, hematological values, biochemistry values, quantitative urinalysis values and organ weights. These tests were conducted at the 5, 1, and 0.1% two-tailed risk level. For multiple comparison, Bartlett's test was used to compare variances among groups at the 5% two-tailed risk level. If the variances were equal, the one-way ANOVA was used followed by Dunnett's or Scheffe's test if significant differences were indicated. If variances were unequal, the Kruskal-Wallis test was used to assess significance, followed by Dunnette's or Scheffe's test if a significant difference among the means was indicated.

C. METHODS

1. Observations

Animals were inspected once daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed at study initiation and weekly thereafter.

3. Food and water consumption, food efficiency, and compound intake

Food consumption for each animal was determined weekly. Mean daily diet consumption was calculated as g food/animal/day and as g/kg bw. Food efficiency (%), calculated as:

[Body Weight Gain (g/animal/day)]/[Food Consumption (g/animal/day)] × 100

and compound intake (mg/kg/day) values were calculated as time-weighted averages from the food consumption and body weight data.

4. Ophthalmoscopic examination

Eyes were examined in all rats in the control and high-dose group at study initiation and at weeks 12-13 of the study.

5. Blood was collected at week 13 (days 85-87) for hematology analysis via puncture of the orbital sinus plexus from animals that were not fasted or anesthetized. Blood was also collected at study termination for clinical chemistry analysis from fasted animals from the carotid artery under anesthesia. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for subchronic studies based on OPPTS 870.3100 Guidelines.

123

b. Clinical chemistry

X	**ELECTROLYTES**	X	**OTHER**
X	Calcium	X	Albumin*
X	Chloride	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus	X	Blood urea nitrogen*
X	Potassium*	X	Total cholesterol*
X	Sodium*		Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total serum protein (TP)*
X	Cholinesterase (ChE)		Triglycerides
X	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)		Phospholipids
X	Serum alanine amino-transferase (also SGPT)*		
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transpeptidase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for subchronic studies based on OPPTS 870.3100 Guidelines.

6. Urinalysis*

Urine samples were collected at weeks 11-12 of the study during a 24-hour fasting period from all rats housed individually in metabolism cages. Water was available *ad libitum*. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
	Sediment (microscopic)		Nitrites
X	Protein	X	Urobilinogen

*Not required for subchronic studies by OPPTS 870.3100 Guidelines

7. Sacrifice and pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for microscopic examination. All tissues preserved from all control and high-dose animals, all tissues and organs showing macroscopic abnormality, target organs from all animals, and the lung, liver, and kidneys from all animals were examined microscopically. In addition, the [XX] organs were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain (several sections)*+
X	Salivary glands*	XX	Heart*+	X	Peripheral. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal glands*+
X	Colon *	XX	Kidneys*+		Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	XX	Parathyroids*
	Gall bladder*	X	Epididymides*+	XX	Thyroids*
X	Pancreas*	X	Prostate*		Zymbal gland
		X	Seminal vesicle*	X	Harderian Gland
	RESPIRATORY	XX	Ovaries*+		
X	Trachea*	X	Uterus*+		OTHER
XX	Lung*	X	Vagina		Bone (femur with joint)
	Nose*			X	Skeletal muscle
	Pharynx*			X	Skin*
	Larynx*			X	All gross lesions and masses*

* Required for subchronic studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

No treatment-related clinical signs of toxicity were observed. Incidences of clinical signs in the treatment groups were comparable to controls.

2. Mortality

All animals survived to study termination.

B. BODY WEIGHT AND WEIGHT GAIN

Treatment with 800 or 1600 ppm 31-1359 resulted in decreased mean absolute body weights and body weight gains in both males and females (see Table 2). Statistically significant decreases in mean absolute body weights were observed in 800-ppm males from weeks 1-12 (90-92% of controls; $p<0.05$; 0.01 except week 11) and in 1600-ppm males from treatment weeks 1-13 (85-87%; $p<0.05$; 0.01). Body weight gains for the treatment period of weeks 1-13 were also decreased in 800- and 1600-ppm males as compared with controls (87 and 80% of controls, respectively), but the difference was statistically significant only for the 1600-ppm males ($p<0.05$). In females, biologically significant decreases in absolute body weights were observed in the 800-ppm group

during weeks 6-13 (89-90%), with the decreases at weeks 6-8 attaining statistical significance ($p<0.05$), and in the 1600-ppm group during weeks 1-13 (77-90%; $p<0.01$). As in males, body weight gains for the treatment period of weeks 1-13 were also decreased in 800- and 1600-ppm females as compared with controls (79 and 59% of controls, respectively), but the difference was statistically significant only for the 1600 ppm females ($p<0.01$). No other statistically significant differences in mean absolute body weights or body weight gains were observed.

TABLE 2. Selected mean absolute body weights (g) and body weight gains (g) for rats fed 31-1359 for 13 weeks

Week	Dose Level (ppm)					
	0	50	100	200	800	1600
Males						
0	178.5±9.1	178.4±8.4	178.4±9.5	178.6±9.8	178.5±8.5	178.8±8.0
1	236.4±13.3	239.1±11.4	236.1±12.7	238.1±10.8	218.6±7.6** (92)[a]	202.4±12.2** (86)
4	370.6±32.0	374.1±20.9	363.5±23.5	371.4±26.2	338.1±17.0* (91)	321.9±21.3** (87)
7	446.2±44.7	439.2±44.6	435.5±26.3	448.0±35.3	400.9±22.1* (90)	380.3±23.3** (85)
10	492.9±56.3	486.1±57.9	487.7±30.8	500.4±39.7	441.9±20.0* (90)	419.6±24.7** (85)
13	506.9±59.7	498.5±85.5	507.6±37.5	525.3±43.2	463.7±24.3 (91)	441.4±28.9* (87)
1-13	328.4±55.4	320.1±88.2	329.1±32.3	346.7±37.9	285.3±27.5 (87)	262.6±24.2* (80)
Females						
0	147.7±7.0	147.9±7.3	147.5±7.4	147.7±7.2	147.5±7.7	147.4±7.4
1	172.8±9.8	175.2±12.0	175.9±9.3	170.9±13.1	169.6±10.6 (98)	155.7±6.6** (90)
4	241.9±25.9	238.9±19.2	235.1±15.0	234.3±20.4	222.0±20.1 (92)	197.3±16.5** (82)
7	278.4±36.3	269.8±25.5	271.3±16.7	275.8±19.5	247.1±22.4* (89)	215.4±18.0** (77)
10	297.6±46.9	287.3±32.0	292.8±17.3	298.4±22.9	264.5±23.2 (89)	232.6±16.2** (78)
13	307.6±49.3	295.0±29.5	304.9±19.1	315.0±28.8	273.6±22.6 (89)	242.5±17.7** (79)
1-13	159.9±44.5	147.1±25.7	157.3±19.5	167.3±26.1	126.1±16.5 (79)	95.0±15.3** (59)

Data taken from Tables 3-1 to 4-2, pp. 40-43, MRID 44651843.
[a] Values in parentheses are percent of control values; calculated by reviewer
Statistically different from controls: *$p<0.05$; **$p<0.01$.

C. FOOD CONSUMPTION, WATER CONSUMPTION, AND COMPOUND INTAKE

1. Food consumption and food efficiency

Mean daily food consumption was decreased in male and female groups administered 800 or 1600 ppm 31-1359 (see Table 4). Decreased food consumption levels (g/animal/day) were observed in 800-ppm males at week 1 (80% of controls; $p<0.01$) and in the 1600-ppm males during weeks 1-7 (78-91% of controls) with the differences attaining statistical significance at weeks 1, 2, and 7 (78, 83, 86% of controls, respectively; $p<0.01$). In females, food consumption (g/animal/day) was decreased in the 800 ppm group at weeks 1-7, 10, 12, and 13 (80-91% of controls; statistically significant at weeks 2 and 3: $p<0.05$; 0.01) and in the 1600 ppm group during weeks 1-13 (73-91% of controls; statistically significant at weeks 1-7 and 11: $p<0.05$; 0.01). Overall food consumption for weeks 1-13 was decreased in 1600-ppm males and females (90 and 81%, respectively, not significant).

The study authors also measured food consumption in terms of g/kg bw. Using this measure, food consumption was statistically decreased in 800-ppm males at week 1 (87%; $p<0.01$), and increased in 800-ppm males at weeks 8 and 13 (113 and 117%, respectively) and in 1600-ppm males at weeks 8 and 10-13 (109-114%; $p<0.05$; 0.01). The only statistically significant change observed in females was a decrease in food consumption in the 800-ppm group at week 2 (85%; $p<0.01$).

TABLE 3. Selected mean food consumption (g/animal/day) for rats fed 31-1359 for 13 weeks

Week	Dose Level (ppm)					
	0	50	100	200	800	1600
Males						
1	23.2±1.5	23.9±2.4	22.8±2.0	23.6±2.4	18.6±2.2** (80) [a]	18.2±1.6** (78)
2	24.1±2.1	25.4±2.2	23.9±2.6	24.6±2.3	22.7±4.5	20.1±1.9** (83)
6	23.3±3.1	24.1±4.4	23.7±2.2	24.6±3.4	23.7±2.3	20.9±1.6 (90)
13	22.0±2.6	23.8±4.3	22.3±2.4	24.7±2.7	23.6±1.6	21.9±2.4
1-13	24.1±1.0	24.7±0.8	23.9±0.8	25.1±0.8	23.2±1.5	21.7±1.5
Females						
1	18.6±4.2	17.7±2.7	17.7±0.8	18.6±4.8	15.9±1.9 (85)	14.1±2.7* (76)
2	19.3±1.6	17.8±2.9	18.5±1.6	17.3±3.0	15.4±2.9** (80)	15.4±1.6** (80)
6	19.2±4.1	19.4±3.8	19.0±1.7	18.3±2.8	16.0±2.3 (83)	14.7±1.4* (77)
13	18.3±4.0	17.0±3.1	18.1±1.7	18.6±2.1	15.5±1.6 (85)	16.7±3.0 (91)
1-13	18.8±0.9	18.2±1.0	18.1±0.9	18.6±0.8	16.4±0.9 (87)	15.2±0.9 (81)

Data taken from Tables 5-1 to 6-2, pp. 44-47, MRID 44651843.
[a] Values in parentheses are percent of control values; calculated by reviewer
Statistically different from controls: *$p<0.05$; **$p<0.01$.

12-7

Mean food efficiency was statistically ($p<0.05$; 0.01) decreased in 1600-ppm males at weeks 1 and 6 (52 and 79% of controls, respectively), and in 1600-ppm females at weeks 1, 3, and 6 (41, 66, and 47% of controls, respectively). Decreases observed in 800-ppm males and females did not attain statistical significance (see Table 4).

TABLE 4. Selected mean food efficiency for rats fed 31-1359 for 13 weeks

Week	Dose Level (ppm)					
	0	50	100	200	800	1600
	Males					
1	35.7±1.9	36.4±2.3	36.2±2.6	36.1±2.6	30.5±6.2 (85)[a]	18.4±4.4** (52)
6	14.8±3.3	12.7±6.4	13.1±2.5	15.4±2.2	13.2±1.6 (89)	11.7±2.1* (79)
13	-2.1±6.0	-2.2±7.0	1.1±3.5	3.1±5.7	3.2±3.3	2.8±4.1
	Females					
1	20.3±6.6	22.1±4.2	22.8±2.9	18.3±7.4	19.7±3.4	8.3±6.2** (41)
6	8.7±3.0	8.1±3.0	8.3±1.8	10.2±3.1	5.2±4.6 (60)	4.1±2.7** (47)
13	2.6±2.6	1.4±5.7	4.6±3.6	4.8±2.2	2.8±2.8	3.2±2.3

Data taken from Tables 9-1 to 10-2, pp. 52-55, MRID 44651843.
[a] Values in parentheses are percent of control values; calculated by reviewer
Statistically different from controls: *$p<0.05$; **$p<0.01$.

2. Water consumption

Water consumption was not measured.

3. Compound consumption

Animals were given the test compound in the diet, and mean daily intake as a time-weighted average (mg compound/kg/day) for both sexes is given in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION

There were no treatment-related ophthalmologic findings in either sex.

E. BLOOD WORK

1. Hematology

No treatment related, statistically significant differences were observed in hematological values in treated animals as compared with controls.

2. Clinical chemistry

Total cholesterol levels were statistically increased in 1600 ppm males (84.6 vs. 60.0 mg/dL for controls = 141% of controls; $p<0.01$) and nonstatistically increased in

128

1600 ppm females (100.0 vs. 80.3 mg/dL for controls = 125% of controls). No other statistically significant differences were observed.

F. URINALYSIS

Urinalysis did not reveal any biologically significant, treatment-related changes. The only statistically significant difference observed was a decrease of urinary ketone bodies in 1600-ppm males.

G. SACRIFICE AND PATHOLOGY

1. Organ weights

Selected mean absolute organ weights and organ weights relative to body weights are presented in Table 5. In 800- and 1600-ppm males, statistically significant increases were observed in absolute (128 and 131% of controls, respectively; $p<0.01$) and relative (129 and 143% of controls, respectively; $p<0.01$) thyroid weights and in relative liver weights (113 and 126% of controls, respectively; $p<0.05$; 0.01). In females, relative liver weights were also statistically increased in 800- and 1600-ppm groups (115 and 128%, respectively; $p<0.01$). Other statistically significant differences ($p<0.05$; 0.01) in organ weights in high-dose females included decreased absolute weights of heart (87%), kidney (right: 90%, n.s.; left: 87%), and adrenals (right and left: 79 and 80%, respectively), and increased relative weights of brain (126%), lung (123%), heart (113%), and kidneys (right and left: 116 and 112%, respectively).

Other statistically significant differences in organ weights that were observed in males but were not biologically significant include: increased relative brain weight (116%), lung weight (112%), left kidney weight (112%) and right and left testes weights (119 and 116%) in the 1600-ppm males and increased relative right and left testes weights in 800-ppm males (113% for both).

129

TABLE 5. Final body weight (g) and selected absolute (g) and relative-to-body (%) organ weights of rats fed 31-1359 for 13 weeks

Organ	Dose Level (ppm)					
	0	50	100	200	800	1600
Males						
Final b.w.	481.3	472.7	483.3	498.3	436.1	413.2* (86) [a]
Liver						
Absolute	12.035	12.931	13.211	13.051	12.289	12.954
Relative	2.486	2.739	2.728	2.614	2.814* (113)	3.139** (126)
Thyroid						
Absolute	0.032	0.038	0.038	0.034	0.041**(128)	0.042** (131)
Relative	0.007	0.008	0.008	0.007	0.009** (129)	0.010** (143)
Females						
Final b.w.	291.7	278.8	287.4	297.6	257.5	225.2** (77)
Liver						
Absolute	7.368	6.906	7.181	7.594	7.515	7.318
Relative	2.535	2.484	2.502	2.551	2.916** (115)	3.248** (128)
Brain						
Absolute	1.979	1.968	1.994	1.998	1.951	1.955
Relative	0.692	0.711	0.697	0.678	0.763	0.874** (126)
Lung						
Absolute	1.164	1.167	1.226	1.179	1.134	1.119
Relative	0.404	0.420	0.427	0.397	0.441	0.498** (123)
Heart						
Absolute	1.003	0.968	1.051	1.042	0.938	0.875* (87)
Relative	0.346	0.348	0.366	0.351	0.365	0.390** (113)
Kidney (R)						
Absolute	0.957	0.931	0.966	1.005	0.947	0.861 (90)
Relative	0.331	0.336	0.337	0.340	0.369* (111)	0.383** (116)
Kidney (L)						
Absolute	0.976	0.905	0.947	1.008	0.934	0.852* (87)
Relative	0.337	0.326	0.330	0.340	0.363	0.378* (112)
Adrenal (R)						
Absolute	0.038	0.041	0.040	0.034	0.033	0.030** (79)
Relative	0.013	0.015	0.014	0.012	0.013	0.013
Adrenal (L)						
Absolute	0.040	0.037	0.040	0.038	0.034	0.032* (80)
Relative	0.014	0.013	0.014	0.013	0.013	0.014

Data taken from Tables 23-1 to 26-3, pp. 81-92, MRID 44651843.
[a] Percentage relative to controls; calculated by reviewer.
Statistically significant; *p<0.05; **p<0.01.

2. Gross pathology

No gross changes that were related to treatment were observed during necropsy.

3. Microscopic pathology

a) Non-neoplastic – The only microscopic change that appeared to be related to treatment was centrilobular hypertrophy of the liver in 800 and 1600 ppm males and females (see Table 6). Other microscopic lesions including microgranulomas of the

liver were either not related to dose or the incidence rates were comparable to controls.

TABLE 6. Incidence and severity of hepatic hypertrophy in rats fed 31-1359 for 13 weeks

Sex	Dose Level (ppm)					
	0	50	100	200	800	1600
Males	0/10 (0) *	1/10 (2.0)	0/10 (0)	0/10 (0)	10/10** (1.8)	10/10** (3.0)
Females	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	8/10** (1.0)	10/10** (1.9)

Data taken from Table 29-1 to 30-3; pp. 97-102, MRID 44651843.
* Data are presented as the number of animals affected/number of animals examined (average grade or description: Grade 1: minimal; 2: mild; 3: moderate; 4: marked)
Statistically significant; **p<0.01.

b) Neoplastic – No neoplastic lesions were identified that were related to treatment.

III. DISCUSSION

A. DISCUSSION

Dietary administration of up to 1600 ppm 31-1359 for 13 weeks did not adversely affect clinical signs, survival rates, hematology, or ophthalmologic findings in males or females.

Treatment with 800 or 1600 ppm 31-1359 adversely affected the growth rate of both male and female rats. The reduced growth rate in high-dose animals was indicated by decreased mean absolute body weights, body weight gain, food consumption, food efficiency, and organ weights. Although the decreases in mean body weights were accompanied by decreases in food consumption, the declining body weights were not just an effect of the decreased food intake as evidenced by the lower food efficiency values observed at various times throughout the study. Overall body weight gain for weeks 1-13 and final mean body weights were statistically decreased in both males and females. Coincident with the body weight decreases in the high-dose females were decreases in mean absolute weights of the heart, kidneys, and adrenals. When considering the organ weights relative to body weights, these same organs in addition to the brain and lungs had increased values relative to controls. These increases in relative weights are most likely the result of decreased final body weights.

The effects of treatment with 800 ppm 31-1359 on body weights and food consumption were bordering on biological and/or statistical significance. The decrements by themselves would normally not be considered an adverse effect of treatment. However, because of the clear dose-response relationship observed in reduced growth, the decrements in this case were considered an adverse effect of treatment. Unlike animals treated with 1600 ppm 31-1359, the decrements in body weights and food consumption in 800 ppm animals were accompanied by statistically nonsignificant decrements in food

131

efficiency and absolute organ weights. It is unclear if unpalatability of the diet was partly responsible for the reduced growth of the 800 and 1600 ppm animals, or whether some other mechanism was responsible for reduced growth. As observed in the high-dose animals, food efficiency was variably decreased in addition to reduced food consumption.

It is uncertain if the dose-related hepatic changes observed in males and females fed 800 or 1600 ppm 31-1359 are adverse. Dose-related increases in relative liver weights in 800 or 1600 ppm-treated animals were accompanied by the presence of hepatic centrilobular hypertrophy as indicated by microscopic examination. The severity of the hypertrophy increased with dose, and was greatest in the high-dose males. High-dose males and females additionally had increased total cholesterol levels coincident with the hepatic hypertrophy. No hepatic changes were evident in males or females fed 50, 100, or 200 ppm 31-1359.

The statistically significant decrease in urinary ketone bodies observed in the urinalysis of 1600 ppm males and the increases observed in absolute and relative thyroid weights in 800 and 1600 ppm males were not considered an adverse effect of treatment because they were not accompanied by other indices of toxicity, such as gross or microscopic pathological changes.

Therefore, 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 gain, and food consumption. The NOAEL for male and female rats is 200 ppm (12.4 and 14.6 mg/kg/day, respectively).

B. STUDY DEFICIENCIES

Minor study deficiencies include that blood clotting measurements were not made during hematology analysis, and the nose, pharynx, and larynx were not collected for microscopic examination. These minor deficiencies did not compromise the results of the study.

Acetamiprid: 13-Week Feeding Study in Mice
Nippon Soda. 1997. MRID No. 44988425

DATA EVALUATION RECORD

ACETAMIPRID
(31-1359)

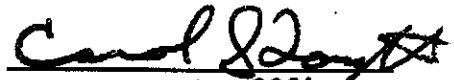
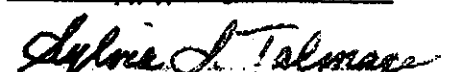
STUDY TYPE: SUBCHRONIC ORAL TOXICITY – MOUSE [OPPTS 870.3100 (82-1b)]
MRID 44988425

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-78D

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	 APR 10 2001
Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	 APR 10 2001
Robert H. Ross, M.S., Group Leader	Signature: Date:	 APR 10 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	 APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

EPA Reviewer: Alan C. Levy, Ph.D. Alan C. Levy, Date AUG. 9, 01
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity – Mouse [OPPTS 870.3100 (§82-1b)]

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: none

TEST MATERIAL: Acetamiprid (99.2% a.i.)

SYNONYMS: 31-1359; (E)-N^1-[(6-chloro-3-pyridyl)methyl)]-N^2-cyano-N^1-methyl-acetamidine

CITATION: Nukui, T. and Ikeyama, S. (1997) Acetamiprid*- Thirteen-week dietary subchronic toxicity study in mice (*Proposed ISO common name, Code No.: 31-1359). Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., 345 Takada, Odawara, Kanagawa, Japan 250-02. Laboratory Project ID: G-0769, September 29, 1997. MRID 44988425, Unpublished.

SPONSOR: Nippon Soda Co., Ltd., 2-2-1 Ohtemachi, Chiyodaku, Tokyo, Japan 100

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 44988425), groups of Crj:CD-1™ (ICR) mice (10 mice/sex/group) were administered 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.

Treatment-related deaths included one 3200-ppm male found dead and another sacrificed moribund during week 12 and two 3200-ppm females which died during weeks 8 and 10, respectively. Clinical signs of toxicity were limited to tremors in 5/10 females in the 3200-ppm group during weeks 4-13. No treatment-related clinical signs were observed in males or the remaining treated females.

Absolute body weights, body weight gains, food consumption, and food efficiency of the 400- and 800-ppm males and females were similar to those of the controls throughout the study. 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. Absolute body weights for the 1600-ppm 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 ($p \leq 0.05$).

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. Food consumption and food efficiency for the 1600-ppm groups were variable with no consistent patterns.

No treatment-related lesions were noted at gross necropsy and no dose-related or biologically significant effects were seen on hematology, urinalysis, or ophthalmologic parameters. Hematological parameters were not measured in the 3200-ppm males and females due to marked growth depression and no test article related changes were observed at lower doses.

In the 1600- and 3200-ppm males and females differences in clinical chemistry parameters, histopathological lesions, and organ weights were indicative of inanition. Glucose was significantly ($p \leq 0.05$ or 0.001) decreased as compared with the controls for the 1600-ppm males (70% of control) and the 3200-ppm males and females (both 40% of control). Total cholesterol was also decreased ($p \leq 0.001$) in the 1600-ppm females (66% of control) and the 3200-ppm males and females (56% and 52%, respectively, of controls). At 3200 ppm, males and females had significant ($p \leq 0.05$ or 0.01) increases in BUN (137% and 178%, respectively), SGPT (157% and 233%, respectively), and SGOT (205% and 180% [n.s.], respectively) as compared with the controls. In the 3200-ppm animals, fat depletion in the adrenal cortex was seen in 4/10 males and 4/8 females (n.s.).

For the 3200-ppm males, absolute lung ($p \leq 0.05$), spleen, and kidney weights ($p \leq 0.001$) were decreased relative to the control group. Relative (to body weight) mean spleen weight was significantly ($p \leq 0.05$) decreased and relative (to body weight) brain, lung, liver, adrenal, and testis weights were significantly ($p \leq 0.01$) increased as compared with the control. For the 3200-ppm females absolute brain, thymus, lung, spleen, kidney, adrenal, and ovary weights were significantly ($p \leq 0.05$ or 0.01) less than those of the controls. Also for the 3200-ppm females, significant ($p \leq 0.05$ or 0.01) differences from the controls were noted for increases in relative brain, lung, liver weights and for decreases in relative spleen and ovary weights. At 1600 ppm significant ($p \leq 0.05$ or 0.01) differences in organ weights included decreased absolute spleen weights for males, increased relative liver and testis weights for males, decreased absolute brain and kidney weights for females, and increased relative liver weights for females. Relative organ weight differences may have been due to lower body weights in treated groups compared with control body weights.

Therefore, 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, decreased glucose and cholesterol levels, and reduced absolute organ weights. The NOAEL for males and females is 800 ppm (106.1 and 129.4 mg/kg/day, respectively).

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

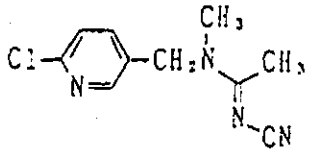
COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, Flagging, and Good Laboratory Practice Compliance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: 31-1359

Description: white crystal
CAS No.: not given
Lot No.: 591001-7
Purity: 99.2% a.i.
Contaminants: none given
Stability: stable for 5 years and 4 months in the dark at -20°C
Structure:



2. Vehicle

Powdered basal diet (MF, Oriental Yeast Co., Ltd., Tokyo) was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: mouse
Strain: Crj:CD-1™ (ICR)
Age and weight at study initiation: approx. 7 weeks: males, 31.2-37.8 g; females, 23.5-28.1 g.
Source: Charles River Japan Inc.
Housing: Animals were individually housed in stainless steel, hanging, wire-mesh cages.
Food: Powdered basal diet (MF, Oriental Yeast Co., Ltd., Tokyo) was available *ad libitum*.
Water: Tap water was available *ad libitum*.
Environmental conditions:
Temperature: 21.7±0.1°C
Humidity: 58.4±2.5%
Air changes: 15/hour

Photoperiod: 12 hour light/12 hour dark
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In life dates

Start: June 4, 1991; end: September 5, 1991

2. Animal assignment

Animal assignment and dose selection are listed in Table 1. Animals were assigned to test groups using a computerized randomization procedure such that mean body weights were comparable between groups.

TABLE 1. Study design

Test group	Dietary Conc. (ppm)	Dose (mg/kg/day)		No. of animals	
		Males	Females	Males	Females
Control	0	0.0	0.0	10	10
Low	400	53.2	64.6	10	10
Mid	800	106.1	129.4	10	10
Mid -high	1600	211.1	249.1	10	10
High	3200	430.4	466.3	10	10

Data taken from text table p. 26 and Text Table II, p. 32, MRID 44988425.

3. Rationale for dose selection

Dietary concentrations were selected on the basis of a preliminary study with the test article in mice. The results were summarized in the current report. Male and female mice were administered 0, 400, 800, or 1600 ppm in the diet for three weeks. No effects were observed at 400 and 800 ppm. Treatment-related effects at 1600 ppm included growth depression (85% of controls in both sexes), decreased food consumption in females, and decreased organ weights in females. Based on the results of this study dietary concentrations of 400, 800, 1600, and 3200 ppm were chosen for the current study.

4. Preparation and analysis of test diets

Test diets were prepared three times during the study at approximately one month intervals and stored in a freezer until use. For each dietary level, an appropriate amount of test article was added to a small amount of diet and ground to a fine powder using a mortar and pestle. The premixes and the required amount of additional diet were transferred to a stainless steel bowl and mixed using a mixer (SS-161, Kanto Kongoki Industrial Co., Ltd., Tokyo) for 7 minutes. Samples taken from the top, middle, and bottom of each diet preparation were analyzed for concentration.

Stability was analyzed for a 50 ppm diet preparation following storage at room temperature for 7 days and in a freezer (-18 to -20°C) for 5 weeks.

Results –
Homogeneity analysis: Concentrations of the test article in samples from the top, middle, and bottom of the diets varied by <10%.

Concentration: Absence of test article was confirmed in the control diets. Concentrations of the test article in the individual samples from all diets were within 6% of nominal.

Stability: Following storage at room temperature for 1 week or frozen for 5 weeks, the mean concentrations of the sample diet were 95% and 103%, respectively, of their initial measured concentrations.

Conclusion: These analyses confirm that the diets were homogeneously mixed, that the initial concentrations of the test article were acceptable, and that the test article was stable in the diets for the duration of use and storage.

5. Statistical analysis

Body weight, food consumption, organ weight, and clinical pathology data were analyzed with Bartlett's test to determine homogeneity of variances. If the variances were equal, data were analyzed by one-way Analysis of Variance (ANOVA); if the variances were unequal, data were analyzed by Kruskal-Wallis test. If a significant difference was indicated, Dunnett's or Scheffe's test was used to determine which means were different from the control. Mortality, clinical observations, ophthalmologic, and macroscopic observations were analyzed by the Chi-square test. The Mann-Whitney U test was used for analysis of semi-quantitative urinalysis values.

C. METHODS

1. Observations

Animals were observed once daily for mortality and moribundity. Detailed physical examinations were conducted weekly on all animals.

2. Body weight

Body weights were recorded on the first day of treatment and weekly during the study period.

3. Food consumption and food efficiency
Food consumption was measured weekly. Food efficiency was calculated as (body weight gain/food consumption) × 100. Compound consumption was calculated from body weight and food consumption data and nominal dietary concentrations.

4. Ophthalmology

Indirect ophthalmic examinations were conducted on the eyes of all mice prior to initiation of treatment and during week 12. A fundus camera was also utilized for the evaluation of the fundus oculi in animals in the control and 3200-ppm groups.

5. Hematology

During week 13, blood was collected for hematology measurements and blood smears from the orbital sinus plexus of all mice except the animals in the 3200-ppm group; animals were not fasted prior to collection. The CHECKED (X) parameters were evaluated:

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	-	Reticulocyte count
	Blood clotting measurements*	-	Blood cell morphology
-	(Activated thromboplastin time)	-	Red cell distribution width
-	(Clotting time)		
-	(Prothrombin time)		

*Required for subchronic studies based on OPPTS 870.3100 Guidelines.
- = not examined

6. Clinical chemistry

At study termination, all surviving animals were fasted for at least 16 hours and blood was collected for clinical chemistry measurements from the cervical vein under ether anesthesia. The CHECKED (X) parameters were examined.

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
-	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein*
X	Cholinesterase (ChE)	-	Triglycerides
X	Creatine phosphokinase	-	Serum protein electrophoresis
X	Lactate dehydrogenase		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
-	Gamma glutamyl transferase (GGT)		
-	Glutamate dehydrogenase		

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.
- = not examined

7. Urinalysis

At weeks 12-13, urine was collected from all survivors during a 24-hourt fast. The CHECKED (X) parameters were measured:

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Urobilinogen
X	Protein	-	Reducing substances

Urinalysis is not required for subchronic studies.
- = not examined

8. Sacrifice and pathology

Animals which died during the study were subjected to gross necropsy. At study termination, after a 16-hour fast, all survivors were sacrificed by exsanguination from the cervical vein while under ether anesthesia and were subjected to gross necropsy. The following tissues (X) were collected from all animals and preserved in 10% phosphate-buffered neutral formalin. In addition, the (XX) tissues were weighed. All tissues from the control and high-dose animals and from animals that died intercurrently and the liver, kidney, and lung from the animals in the lower dose groups were examined microscopically. All gross lesions from any animal were examined microscopically.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
-	Oral tissues	X	Aorta*	XX	Brain*+
-	Tongue	X	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary
X	Stomach*	XX	Spleen*	X	Eyes (optic n.)
X	Duodenum*	XX	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Harderian gland
X	Rectum*	XX	Testes*+	X	Mammary gland*
XX	Liver*+	X	Epididymides*	X	Parathyroids*
X	Gallbladder	X	Prostate*	X	Thyroids*
X	Pancreas*	X	Seminal vesicle*	-	Coagulation glands
		XX	Ovaries*		
	RESPIRATORY	X	Oviducts		OTHER
X	Trachea*	X	Uterus*	X	Bone*
XX	Lung*	-	Cervix	X	Skeletal muscle*
-	Nose (nasal turbinates)	X	Vagina	X	Skin*
-	Pharynx			X	All gross lesions and masses*
-	Larynx				

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.
- = not examined

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

During week 12, one 3200-ppm male was found dead and another was sacrificed moribund due to marked weight loss. Two 3200-ppm females died, one each during weeks 8 and 10. In addition, one control female and one 800-ppm female died during blood collection, but these two deaths are not considered to be treatment-related. All other animals survived to scheduled sacrifice. Clinical signs of toxicity were limited to tremors in 5/10 females in the 3200-ppm group during weeks 4-12. No treatment-related clinical signs were observed in males or the remaining treated females.

B. BODY WEIGHTS AND BODY WEIGHT GAINS

Selected mean body weights and body weight gains of males and females are listed in Table 2. Absolute body weights and body weight gains of the 400- and 800-ppm males and females were similar to those of the controls throughout the study. Absolute body weights for the 3200-ppm males and females were significantly ($p \leq 0.01$ or 0.001) less than the controls beginning at week 1. For the 3200-ppm males and females, absolute body weights during the study were 65-79% and 64-77%, respectively, of the control group levels. 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. Absolute body weights for the 1600-ppm males and females were significantly ($p \leq 0.05$ or

0.01; 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 ($p \leq 0.01$ or 0.001).

TABLE 2: Selected body weights and body weight gains of male and female mice administered 31-1359 in the diet for 13 weeks (g) - initially 10/sex/group

Week of study	0 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm
Males					
0	34.26	34.45	34.20	34.48	34.46
1	35.06	35.24	34.88	32.85	27.56** (79)[a]
3	36.75	36.40	36.03	33.54* (91)	27.45** (75)
5	38.29	37.55	37.49	34.41* (90)	27.09** (71)
7	39.80	39.48	38.84	35.74* (90)	27.13** (68)
9	40.84	41.17	39.92	36.22* (89)	27.31** (67)
11	41.78	42.25	41.04	36.60* (88)	27.19** (65)
13	41.22	41.71	40.13	35.83** (87)	27.34** (66)
Wt. gain 1-13	6.96	7.26	5.93	1.35** (19)	-7.07***
Females					
0	25.78	25.77	25.89	25.84	25.73
1	26.62	26.84	26.82	24.20** (91)	20.62** (77)
3	28.34	28.18	27.77	25.68** (91)	20.81** (73)
5	29.85	28.86	30.11	26.48** (89)	20.86** (70)
7	30.77	29.82	31.12	27.36** (89)	21.22** (69)
9	31.53	30.24	30.96	27.89** (88)	21.47*** (68)
11	33.54	31.17	31.49	27.49** (82)	21.55***(64)
13	33.64	30.73	31.37	27.45* (82)	21.61*** (64)
Wt. gain 1-13	7.74	4.96	5.46	1.61*** (21)	-4.25***

Data taken from Tables 3 and 4, pp. 40-41 and 42-43, respectively, MRID 44988425.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$
DIED: males = 3200 ppm wk 12, 3200 ppm wk 12; females = 0 ppm wk 13 (accident), 800 ppm wk 13 (accident), 3200 ppm wk 8 and 10.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption and food efficiency

Selected food consumption data are given in Table 3. Food consumption by the 400- and 800-ppm males and females was similar to that of the controls throughout the study. Males and females in the 1600 ppm group had slightly lower food consumption as compared with the controls at each weekly interval with statistical significance attained only once in females. 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. Overall food consumption by the 3200-ppm males was only 72% of the controls. Food consumption by the 3200-ppm females was significantly ($p \leq 0.01$; 65-73% of controls) less than that of the controls throughout the study resulting in overall food consumption 65% of the control group level.

Food efficiency by the 400- and 800-ppm males and females was generally similar to 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. Food efficiency values for the 1600-ppm males and females were occasionally less than or greater than ($p \leq 0.05$) those of the controls.

TABLE 3: Selected food consumption (g/day) of male and female mice administered 31-1359 in the diet for 13 weeks

Week	0 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm
Males					
1	4.4	4.4	3.9	3.9	2.8** (64)[a]
3	4.8	5.2	5.2	4.6	4.3
6	5.1	5.4	5.2	4.7	3.6** (71)
9	5.3	5.4	5.2	4.8	4.0** (75)
13	5.2	5.4	5.3	4.7	3.8***(73)
1-13 (mean)	5.0	5.2	5.1	4.6	3.6 (72)
Females					
1	4.4	4.3	4.3	4.0	2.9** (66)
3	5.0	5.0	4.9	4.1* (82)	3.4** (68)
6	4.4	4.9	4.6	4.2	3.2* (73)
9	5.1	5.0	5.1	4.6	3.3** (65)
13	4.9	4.7	5.1	4.4	3.6** (73)
1-13 (mean)	4.8	4.7	4.8	4.1	3.1 (65)

Data taken from Tables 5 and 6, pp. 44-45 and 46-47, respectively, MRID 44988425.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

2. Compound intake

Overall time-weighted average doses are shown in Table 1.

D. OPHTHALMOLOGY

No ophthalmologic lesions were observed in any animal after 12 weeks of treatment with the test article.

E. HEMATOLOGY

Hematological parameters were not measured in the 3200-ppm males and females due to marked growth depression. In the other groups, no differences in any hematological parameter were noted between the treated and control groups of either sex with one exception. Females in the 1600 ppm group had significantly ($p \leq 0.05$) reduced hemoglobin to 94% of the control level.

F. CLINICAL CHEMISTRY

Selected clinical chemistry values are shown in Table 4. Glucose was significantly ($p \leq 0.05$ or 0.001) decreased as compared with the controls for the 1600-ppm males and the 3200-ppm males and females. Total cholesterol was also decreased ($p \leq 0.01$) in the 800- and 1600-ppm females and the 3200-ppm ($p \leq 0.001$) males and females as compared with the controls. The 3200-ppm males and females had significant ($p \leq 0.05$ or 0.01)

increases in BUN, SGPT, and SGOT (n.s. for females) activities as compared with the controls. In addition, the 3200-ppm males had increased ($p \leq 0.05$) cholinesterase activity as compared with the control values.

TABLE 4: Selected clinical chemistry parameters of male and female mice administered 31-1359 in the diet for 13 weeks

Endpoint	0 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm
Males					
Glucose (mg/dL)	166.7	158.6	160.8	117.0* (70)[a]	66.8*** (40)
BUN (mg/dL)	33.0	34.8	33.8	34.0	45.2* (137)
Cholesterol (mg/dL)	142.7	156.5	143.9	114.3	80.1*** (56)
SGPT (IU/L)	21	22	20	20	33* (157)
SGOT (IU/L)	59	61	70	82	121*** (205)
Cholinesterase (IU/L)	5819	5202	4980	5729	7768* (133)
Females					
Glucose (mg/dL)	95.2	104.3	109.0	83.9 (88)	38.5* (40)
BUN (mg/dL)	24.7	35.6	27.8	29.8	43.9*** (178)
Cholesterol (mg/dL)	109.4	97.4	81.2** (74)	72.5** (66)	56.8*** (52)
SGPT (IU/L)	21	28	25	30	49*** (233)
SGOT (IU/L)	69	114	93	108	124
Cholinesterase (IU/L)	8795	9638	9234	7946	9487

Data taken from Tables 19 and 20, pp. 68-70 and 71-73, respectively, MRID 44988425.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.; ***$p \leq 0.001$

G. URINALYSIS

No treatment-related differences were observed in urinalysis parameters between the treated and control rats of either sex. Males in the 3200-ppm group had significantly ($p \leq 0.01$) lower urinary pH as compared with that of the controls (5.71 ± 0.28 S.D. vs 6.24 ±0.21 for the controls).

H. SACRIFICE AND PATHOLOGY

1. Gross pathology

No treatment-related lesions were noted at necropsy.

2. Organ weights

Selected organ weight data are given in Table 5. Terminal body weights of the 1600- and 3200-ppm males and females were significantly ($p \leq 0.01$ or 0.001) less than the controls. For the 3200-ppm males: absolute - decreased thymus ($p \leq 0.05$), lung, spleen and kidney; relative to body weight - increased ($p \leq 0.01$) brain, lung, liver, adrenal and testis. For the 3200-ppm females: absolute - decreased ($p \leq 0.05$, 0.01 or 0.001) brain, thymus, lung, spleen, kidney, adrenal and ovary; relative to body weight - increased brain, lung and liver plus decreased spleen and ovary. At 1600 ppm, males had increased ($p \leq 0.05$, 0.01 or 0.001) absolute liver and testis weights; females, decreased absolute brain and kidney with increased relative liver weights. At

400 or 800 ppm, there was increased ($p \leq 0.05$) relative liver weight in the 800 ppm group only. For the 3200-ppm group, organ weight differences from control most likely were due to lower terminal body weights.

TABLE 5: Selected organ weight data from male and female mice administered 31-1359 in the diet for 13 weeks

Organ	0 ppm	400 ppm	800 ppm	1600 ppm	3200 ppm
Males					
Final Body Wt. (g)	36.62	36.93	35.63	31.72** (87)[a]	24.04*** (66)
Brain					
absolute (g)	0.511	0.508	0.515	0.488	0.472
relative to b.wt.	1.408	1.385	1.457	1.542	1.985*** (141)
Liver					
absolute (g)	1.464	1.550	1.580	1.468	1.323
relative to b.wt.	4.004	4.209	4.452* (111)	4.627** (116)	5.509*** (138)
Lung					
absolute (g)	0.212	0.209	0.214	0.202	0.179* (84)
relative to b.wt.	0.581	0.567	0.603	0.638	0.751*** (129)
Spleen					
absolute (g)	0.087	0.079	0.083	0.072* (83)	0.044*** (51)
relative to b.wt.	0.239	0.213	0.235	0.228	0.180* (75)
Right Kidney					
absolute (g)	0.304	0.326	0.322	0.280	0.218*** (72)
relative to b.wt.	0.834	0.886	0.907	0.878	0.910
Left Kidney					
absolute (g)	0.302	0.325	0.307	0.269	0.212*** (70)
relative to b.wt.	0.826	0.885	0.866	0.844	0.889
Females					
Final Body Wt. (g)	30.42	26.75	27.43	24.51** (81)	19.18*** (63)
Liver					
absolute (g)	1.247	1.135	1.272	1.304	1.226
relative to b.wt.	4.093	4.250	4.604* (112)	5.311*** (130)	6.404*** (156)
Brain					
absolute (g)	0.521	0.510	0.523	0.485* (93)	0.441*** (85)
relative to b.wt.	1.744	1.928	1.923	1.994	2.310*** (132)
Lung					
absolute (g)	0.195	0.183	0.189	0.183	0.156*** (80)
relative to b.wt.	0.650	0.690	0.691	0.749	0.819*** (126)
Spleen					
absolute (g)	0.089	0.080	0.076	0.063	0.034*** (38)
relative to b.wt.	0.297	0.299	0.274	0.256	0.175** (59)
Right Kidney					
absolute (g)	0.223	0.212	0.221	0.182** (82)	0.158*** (71)
relative to b.wt.	0.742	0.798	0.810	0.749	0.830
Left Kidney					
absolute (g)	0.213	0.210	0.211	0.181** (85)	0.149*** (70)
relative to b.wt.	0.706	0.790	0.773	0.742	0.783

Data taken from Tables 23-26, pp. 83-90, MRID 44988425.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

3. Microscopic pathology

Centrilobular hepatocellular hypertrophy was observed in 8/10 males and 9/10 females (both sexes $p \leq 0.01$) administered 3200 ppm compared with none of the animals in the control or other treated groups. Also observed only in 3200-ppm animals, fat depletion in the adrenal cortex was seen in 4/10 males and 4/8 females (n.s.). In addition for the 3200-ppm groups, significantly ($p \leq 0.05$) decreased incidence rates occurred for lipofuscin pigmentation in the adrenal cortex of males (1/10 vs 7/10 controls) and microgranuloma in the liver of females (2/10 vs 8/10 controls). In females, diffuse fatty degeneration in the liver was observed in 4/10, 2/10, 5/10, 9/10 ($p \leq 0.01$), and 6/10 animals in the 0-, 400-, 800-, 1600-, and 3200-ppm groups, respectively.

III. DISCUSSION

A. AUTHORS' CONCLUSIONS

Five/ten females of the 3200 ppm group showed tremor at weeks 4-13; two died, one at week 8 and one at week 10. One control and one 800 ppm females died due to sampling accidents (week 13 hematology). Two males of the 3200 ppm group died week 12; one in extremis due to decreased body weight. No tremors in males.

Decreased body weights were noted in both sexes of the 1600 and 3200 ppm groups at the study termination. The decreases in food consumption values were noted in both sexes of 3200 ppm groups and in females of the 1600 ppm group. Food efficiency was decreased in both sexes of the 3200 ppm groups.

No ophthalmic effects were noted. Hemaglobin values were decreased slightly with statistical significance in 1600 ppm females. Hematology parameters not measured in the 3200 ppm groups due to marked decreases in body weight gains. Statistically significant decreases of total cholesterol and glucose concentration were seen in both sexes of the 800 ppm and above groups. Statistically significant increases were noted in BUN of both sexes at 3200 ppm (no effects in creatinine). A statistically significant decrease in urinary pH was found in the 3200 ppm group males at week 12 examination.

Statistically significant increases were noted in the liver/body weight ratios of males and females at 800 ppm and above. In 1600 and 3200 ppm groups, decrease in weights were found for many organs and were considered to be attributed to the decreased body weights of the groups. No grossly observable necropsy findings were noted. Microscopically, dose-related centrilobular hepatocellular hypertrophy was seen in males and females of the 3200 ppm groups. In animals that died on the study, pulmonary congestion, thymic atrophy, as well as some lesions seen in terminally sacrificed animals, were observed.

Based on the results mentioned above, the effects of the test compound were tremor, decreased body weight gains, decreased food consumption, decreased hemoglobin concentrations, decreased serum total cholesterol and glucose levels, decreased urinary

pH, increased liver/body weight ratios and centrilobular hepatocellular hypertrophy. The NOEL was considered to be 400 ppm (53.2 and 64.6 mg/kg/day, respectively) in males and females.

B. DISCUSSION

Deaths of two males (week 12) and two females (weeks 8 and 10) in the 3200-ppm groups were considered treatment-related. Clinical signs of toxicity were observed only in the 3200-ppm females and consisted of tremors (5/10 mice during weeks 4-13) which did not correspond to inhibition of serum cholinesterase activity.

The main effect of test article administration was marked growth suppression at 3200 ppm and reduced growth at 1600 ppm. The 3200-ppm animals had a net body weight loss during the study while body weight gains by the 1600-ppm animals were greatly reduced as compared with the controls. Food consumption was also reduced at both of these dietary concentrations. However, the magnitude of the decreases in food consumption were not sufficient to account for the effects on body weights as indicated by food efficiency values. Food efficiency values were often negative for the 3200-ppm groups and variable for the 1600-ppm group.

Hematology parameters were not measured in the 3200-ppm animals due to their marked weight loss. The reviewer agrees with the study authors that blood collection would have probably been too great a stress on these animals and might have caused several deaths. No treatment-related changes in hematology were observed in the other groups. Therefore, the lack of this data for the 3200-ppm group does not compromise the integrity of this study.

Differences in clinical chemistry parameters are indicative of inanition in the 1600- and 3200-ppm groups. Large decreases in glucose and cholesterol levels in both groups suggest that the animals were starving. In support of this, increased BUN, SGPT, and SGOT in the 3200-ppm males and females indicate muscle breakdown. Decreased cholesterol in the 800-ppm females was not associated with effects on body weight and therefore, not considered an adverse effect.

At terminal sacrifice, reduced absolute organ weights of animals in the 1600- and 3200-ppm groups were probably a result of the extreme growth suppression observed in these animals. Consequently increased relative organ weights were considered a result of lower final body weights of these animals. On the other hand, lower relative spleen weights were probably due to the markedly reduced absolute weights for this organ.

Fat depletion observed in the adrenal cortex from 3200-ppm males and females is also considered to be due to inanition. Other findings at microscopic examination were not considered adverse (hepatocellular hypertrophy) or the relationship to treatment is uncertain.

Therefore, 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, decreased glucose and cholesterol levels, and reduced absolute organ weights. The NOAEL for males and females is 800 ppm (106.1 and 129.4 mg/kg/day, respectively).

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

C. STUDY DEFICIENCIES

No deficiencies were noted in the conduct of this study.

DER #9

Acetamiprid: 13-Week Feeding Study in Dogs
Nippon Soda. 1998. MRID No. 44988424

Reviewer: Gordon Cockell, Date June 20, 2001

STUDY TYPE: Subchronic Oral Toxicity, Dietary - Dog; OPPTS 870.3150 [§82-1]; OECD 409.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.46%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Auletta, C.S. (1998) A subchronic (3-month) oral toxicity study of NI-25 in the dog via dietary administration. Bio/dynamics, Inc. East Millstone, NJ. Study no. 91-3727. June 30, 1998. MRID No. 44988424. Unpublished.

SPONSOR: Nippon Soda, Tokyo, Japan

EXECUTIVE SUMMARY: 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).

This subchronic toxicity study is classified as acceptable and it satisfies the guideline requirement for a subchronic oral study (82-1); OECD 409 in the dog.

COMPLIANCE: Signed and dated GLP, QA and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** NI-25 (Acetamiprid)

Description:	pale yellow powder
Lot/Batch #:	NNI-02
Purity:	99.46 % a.i.
Compound Stability:	Stable for 2 months in the dark at 50°C
CAS #:	135410-20-7

2. **Test animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Approximately six months, males 8.6 kg (7.6-10.0), females 8.0 kg (7.3-8.6)	
Source:	Marshall Farms, U.S.A., Inc.	
Housing:	Individual, in elevated metal grid cages. Animals were provided with exercise according to Animal Welfare Standards, following Bio/Dynamics Standard Operating Procedures	
Diet:	Standard laboratory diet (Purina Certified Canine Meal Diet #5007), 400 g/animal/day, available for 22 hours per day.	
Water:	Tap water, available *ad libitum*	
Environmental conditions:	**Temperature:**	20-26°C
	Humidity:	35-84%
	Air changes:	Not stated
	Photoperiod:	12 hour light/dark cycle (7 am - 7 pm via automatic timer)
Acclimation period:	Approximately 4 weeks	

B. STUDY DESIGN:

1. **In life dates - Start:** May 22, 1992 **End:** August 25, 1992

2. **Animal assignment:** Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw/day)	# Male	# Female
Control	0	0	4	4
Low	320	13/14	4	4
Mid	800	32/32	4	4
High	2000	58/64	4	4

3. **Diet preparation and analysis:** Appropriate amounts of test substance were mixed with the Certified Diets to achieve the desired concentrations. Fresh diets were prepared weekly. Homogeneity analyses were conducted on mock batches of the low- and high-concentration diets prior to initiation of dosing. Three samples were taken from the top, middle and bottom sections of each dietary batch. Stability of the test substance in the dietary mixture was demonstrated in the 4-week range-finding study. In that study, stability was demonstrated after storage of test diets at room temperature for 15 days.

Concentration analysis of test diets was conducted weekly for the first 4 weeks and monthly thereafter to ensure that the diets were prepared at their intended concentrations.

Results - Homogeneity Analysis: The homogeneity of the test diets ranged from -1% to +6% of the mean concentrations, for samples taken from the top, middle and bottom of the mixture.

Stability Analysis: In the range-finding toxicity study in dogs, NI-25 was found to be stable in test diets when stored at room temperature over a period of 15 days. After 15 days of storage at room temperature, concentrations ranged from 89.6% to 103% of nominal concentrations.

Concentration Analysis: The mean test material concentration in prepared diets were 103%, 105% and 98.2% of nominal concentrations for the 320, 800 and 2000 ppm dose groups, respectively.

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

4. **Statistics** - Statistical evaluation of equality of means was made by the appropriate one way analysis of variance technique, followed by a multiple comparison method if needed. First, Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric methods were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a non-parametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

   A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the non-parametric case, Jonckheere's test for monotonic trend was used.

   The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other tests were conducted at the 5% and 1%, two-sided risk level.

C. METHODS:

1. **Observations:** Animals were inspected at least twice daily for signs of toxicity and mortality. Detailed physical examinations were conducted weekly

2. **Body weight:** Animals were weighed twice pretest, weekly during the treatment period and at study termination after fasting.

3. **Food consumption and test article intake:** Food consumption for each animal was measured and recorded daily (seven days per week) and reported as weekly means. Nominal test article intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption and body weight data.

4. **Ophthalmoscopic examination:** All animals were examined pretest and at study termination. Eyelids, lacrimal apparatus and conjunctiva were examined grossly; cornea, anterior chamber, lens, vitreous humor, retina and optic disc were examined by indirect ophthalmoscopy. Eyes were examined after installation of Mydriafair 1%.

5. **Haematology & clinical chemistry:** Blood was collected from all animals pretest, at week 7 and at study termination (week 13) for haematology and clinical biochemical analysis. Blood was obtained from unanesthetized animals via the jugular vein. Animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. Haematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
X	Plasma Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	A/G ratio
X	Serum alanine amino-transferase (also SGPT)*		Phospholipids
X	Serum aspartate amino-transferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic studies based on Subdivision F Guidelines

6. **Urinalysis:** Urine was collected from water-deprived animals (approximately 2 hours) pretest and at weeks 7 and 13. Urine volume was measured over a 16-hour interval. Animals were food and water-deprived for this interval. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood / blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for subchronic non-rodent studies based on Guideline 870.1350

7. Sacrifice and Pathology: Gross postmortem examinations were conducted on all animals and the CHECKED (X) tissues were collected for histopathological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	X	Heart*	X	Periph. nerve (sciatic)*
X	Esophagus*	X	Sternum with bone marrow*	X	Spinal cord (3 levels)^{T}
X	Stomach*	X	Lymph nodes (mesenteric,	X	Pituitary*
X	Duodenum*		submandibular)*	X	Eyes (optic n.)^{T}
X	Jejunum*	X	Spleen*		
X	Ileum*	X	Thymus*		GLANDULAR
X	Cecum*			XX	Adrenal gland*
X	Colon*		UROGENITAL		Lacrimal gland^{T}
X	Rectum*	XX	Kidneys*+	X	Mammary gland^{T}
XX	Liver*++	X	Urinary bladder*	XX	Parathyroids*++
X	Gall bladder*	XX	Testes*+	XX	Thyroids*++
X	Pancreas*	XX	Epididymides		
		X	Prostate		OTHER
	RESPIRATORY		Seminal vesicle	X	Skeletal muscle
X	Trachea*	X	Ovaries	X	Skin
X	Lung*	X	Oviducts	X	All gross lesions and masses*
	Nose		Vagina		
	Pharynx	X	Uterus*		
	Larynx				

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. **Clinical signs of toxicity and physical examinations** - There were no treatment-related clinical signs recorded during the study and physical examinations did not reveal any effect of treatment.

2. **Mortality** - All animals survived throughout the study.

B. Body weight and body weight gain: All high dose males and females lost weight during the first week of the study, and all but one (male) lost weight from week 1 to 2. Body weight changes among high dose animals remained around zero for the remainder of the study. Statistically significant changes were apparent among high dose females throughout the study period, however, among males, the differences were only statistically significant for the cumulative body weight gain data at weeks 10, 11 and 13. Group mean body weight and body weight gain were slightly decreased, relative to controls at 800 ppm during the first 4 weeks of the study period, and were generally similar to control values for the remainder of the study. The only difference that was statistically significant at this dose was the cumulative body weight gain among females at week 4. Body weight and body weight gain in males and females at 340 ppm was similar to control values throughout the study period. The study author did not consider the changes observed at 800 ppm to represent adverse, treatment-related effects. The total body weight gain among mid-dose males was 29% of

controls and in mid-dose females, total body weight gain over the study period was 67% of controls. Based on the magnitude of the observed reduction in body weight gain at this dose, the reviewer considers these findings adverse, treatment-related effects.

TABLE 2: Group mean body weight and body weight gain over the treatment period

Dose rate (ppm)	Body Weights (g)						Total Weight Gain week 0-13 (kg)
	Week 0	Week 1	Week 3	Week 7	Week 10	Week 13	
Male							
0	8.6	8.8	9.2	10.0	10.6	10.8	2.1
320	8.7	8.8	9.1	9.2	9.8	9.9	1.3
800	8.6	8.7	8.9	9.1	9.4	9.2	0.6
2000	8.7	8.3	8.0	8.3	8.4	8.6	-0.1*
Female							
0	8.0	8.3	8.6	9.0	9.0	8.9	0.9
320	8.0	8.4	8.6	9.1	9.2	9.1	1.1
800	8.0	8.0	8.0	8.5	8.7	8.6	0.6
2000	8.0	7.5	7.1*	7.0**	7.2**	7.1**	-0.9**

Data extracted from page 62-68 of the study report
* Significantly different from control ($p<0.05$)
** Significantly different from control ($p<0.01$)

C. Food consumption and compound intake:

1. Food consumption - The pattern of food consumption was consistent with the observed changes in body weight and body weight gain. A marked decrease in food consumption was observed at 2000 ppm. Group mean food consumption was significantly lower than controls for high dose males during weeks 1 and 2, and remained slightly lower than controls until week 7. High dose females had significantly lower food consumption for weeks 1 through 6, with slightly lower values recorded for most of the remainder of the study. Mid dose females had slightly reduced food consumption for the first three weeks of the study. Food consumption among mid dose males and low dose males and females was generally similar to controls.

2. Compound consumption - Mean compound consumption is shown in Table 3, below.

TABLE 3: Mean test article intake (mg/kg bw/day)

Dietary concentration (ppm)	0	320	800	2000
Males	0	13	32	58
Females	0	14	32	64

D. Ophthalmoscopic examinations: There were no observations at the terminal ophthalmoscopic examination that were attributed to treatment with acetamiprid.

E. Blood analyses:

1. Haematology - There were no changes in hematological parameters that were attributed to treatment with acetamiprid. A slight prolongation in activated partial thromboplastin time (APTT) was

reported for high dose males at 1.5 and 3 months and for mid dose males at 3 months and a slight prolongation in prothrombin time was observed in high dose females at 3 months. No significant difference in APTT was noted among females at any time during the study, no effect on prothrombin time was apparent in males and the only change in APTT that was statistically significant was the observation among males at 3 months. The study author reported that individual values were generally within normal ranges and the toxicological significance of this observation is questionable. In the opinion of the reviewer, the observed differences are probably incidental.

TABLE 4: Selected hematology results

Dose (ppm)	0	320	800	2000
Males				
Prothrombin time (sec)				
- pretest	5.5±0.1	5.8±0.1*	5.5±0.1	5.5±0.1
- 7 weeks	5.6±0.2	5.7±0.2	5.6±0.1	5.7±0.2
- 13 weeks	5.7±0.1	5.8±0.2	5.8±0.2	5.8±0.2
Activated partial thromboplastin time (sec)				
- pretest	9.4±0.5	9.4±0.3	9.6±0.4	10.1±0.5
- 7 weeks	9.4±0.3	9.8±0.2	9.9±0.7	11.3±0.8**
- 13 weeks	9.8±0.6	10.1±0.4	11.4±2.5	11.7±1.6
Females				
Prothrombin time (sec)				
- pretest	5.6±0.0	5.6±0.0	5.6±0.4	5.7±0.1
- 7 weeks	5.7±0.1	5.4±0.2	5.6±0.1	5.8±0.2
- 13 weeks	5.6±0.0	5.6±0.0	5.7±0.1	6.0±0.2**
Activated partial thromboplastin time (sec)				
- pretest	9.8±0.6	9.6±0.2	9.6±0.3	9.5±0.4
- 7 weeks	9.9±0.8	10.0±0.3	10.0±0.2	11.0±0.7
- 13 weeks	10.7±2.0	10.9±1.1	10.6±0.8	10.7±0.5

Data obtained from pages 76-82 of the study report
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

2. Clinical Chemistry - There were no changes in clinical biochemistry parameters that could be attributed to treatment with acetamiprid.

F. Urinalysis: Examination of the urinalysis data revealed no evidence of any treatment-related effects.

G. Sacrifice and Pathology:

1. Organ weight: A dose-related, statistically significant decrease in mean absolute thyroid/parathyroid weight was observed in all treated female groups. Group mean thyroid/parathyroid weight relative to body weight was also reduced in all treated female groups as well as high dose thyroid/parathyroid relative to brain weight. No change in this organ weight was observed among males, and the observed changes were within the range observed in historical controls. In the absence of morphologic changes in the thyroid/parathyroid, the study author stated that the toxicological significance of this observation is not clear. As noted in table 5, below, the data appear to be skewed by a slightly higher than normal mean value for the concurrent control animals, hence, in the opinion of the reviewer, the observed differences are considered incidental.

Group mean testes/epididymides weights were slightly lower than controls for all treated males, due primarily to one low value in each of the treated groups. The differences were not statistically significant and were considered to reflect normal biological variation. In addition, the organ weights relative to brain and body weight were similar to control values. There were no other noteworthy organ weight changes observed in the study.

TABLE 5: Selected organ weight data

Dose (ppm)	0	320	800	2000
Thyroid/parathyroid weight (♀)	0.857±0.072	0.679±0.108*	0.665±0.037**	0.550±0.068**
Historical control - mean (range)	0.703 (0.493-1.006)			
Thyroid/parathyroid weight (♂)	0.730±0.194	0.793±0.172	0.808±0.228	0.733±0.120
Thyroid/parathyroid relative to body weight (♀)	10.50±1.15	7.90±1.18	7.99±0.50	7.92±1.17
Thyroid/parathyroid relative to brain weight (♀)	12.11±0.81	9.84±1.75	10.09±0.71	7.66±1.25**
Testes/epididymides weight	18.0±3.8	15.8±8.5	13.1±3.8	12.3±3.9

Data obtained from pages 92-98 of the study report
* Significantly different from control, $p<0.05$
** Significantly different from control, $p<0.01$

2. Gross pathology: There were no macroscopic changes observed at necropsy that were attributed to treatment with acetamiprid.

3. Microscopic pathology: There were no microscopic changes that were attributed to treatment with acetamiprid.

III. DISCUSSION

A. Investigators' conclusions:

Conclusions from the pathology report:

"1. All of the animals on test survived to the end of the 90 day test period when they were killed and examined postmortem.

"2. There were no macroscopic morphologic findings which were considered to be related to the dietary administration of NI-25.

"3. There were no microscopic morphologic findings which were considered to be related to the dietary administration of NI-25."

Conclusions from the main study report:

"Although decreased thyroid/parathyroid weights were seen for females at all dose levels (320, 800 and 2000 ppm), the toxicological significance of this finding, in the absence of morphological alterations, is not clear. Based on all other evaluations, the no observed effect level (NOEL) for dietary administration of NI-25 to dogs for 3 months under conditions of this study was 800 ppm (32 mg/kg/day)."

B. Reviewer comments: In a subchronic (13-week) oral toxicity study in Beagle dogs, acetamiprid was administered in the diet at nominal concentrations of 0, 320, 800 or 2000 ppm, equal to daily average intakes over the study period of 0, 13, 32 and 58.0 mg/kg bw/day in males and 0, 14, 32 and 64 mg/kg bw/day in females.

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).

C. Study deficiencies: None.

DER #10

Acetamiprid: Acute Neurotoxicity Study in Rats
Rhone-Poulenc Secteur Agro. 1997. MRID No. 44651842 and 44651844

PMRA Reviewer: Scott Hancock, Date: June 6, 2001
Secondary Reviewer: Gordon Cockell, Date: July 13, 2001

STUDY TYPE: Acute Neurotoxicity - Rats OPPTS 870.6200; OECD 424.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical) 99.9%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Hughes, E.W. (1997) Acetamiprid Neurotoxicity to Rats by Acute Oral Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number RNP/509/970851, November 3, 1997. MRID # 44651842, Unpublished.

SPONSOR: Rhone-Poulenc Secteur Agro, France

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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** Acetamiprid

Description:	pale yellow powder
Lot/Batch #:	NFG-02
Purity:	99.9% a.i.
CAS #:	160430-64-8

2. **Vehicle and/or positive control:** 0.5% sodium carboxymethylcellulose

3. **Test animals:**

Species:	rat	
Strain:	Crl:CD-BR	
Age/weight at dosing:	45 days/ males (199-205g), females (154-158g)	
Source:	Charles River Breeding Laboratories, Kent, England	
Housing:	individually in stainless steel mesh cages	
Diet:	SDS Rat No. 1 Maintenance diet *ad libitum*	
Water:	tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 1°C
	Humidity:	50 ± 10%
	Air changes:	not noted
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	10 days for males and 17 days for females	

B. STUDY DESIGN:

1. **In life dates:** **Start:** January 6, 1997 **End:** February 7, 1997

2. **Animal assignment and treatment:** Animals were assigned to the test groups noted in Table 1 using a stratified randomization process so that body weight means were similar for each group. Rats were given a single oral dose by gavage then observed daily and weighed on test days 1, 7, and 14. Administration was staggered over a 2-day interval to facilitate neurobehavioural observations. Food consumption was determined on a weekly basis. Survivors were sacrificed and a necropsy was performed on day 15.

3. **Dose selection rationale:** The timing of the FOB measurements on the day of dosing, and the dose selection were based on a dose range finding study (laboratory report # RNP/510/970145). Male and female Crl:CD-BR rats (3/sex/dose) were given acetamiprid by gavage at doses of 10, 50, and 100mg/kg bw and were observed for 14 days. FOB measurements were performed prior to dosing, ½, 2, and 5 hours post dosing to determine the time of maximum effect of dosing. The results indicated clinical signs of neurotoxicity in both males and females at doses of 50 and 100mg/kg and the time of peak effect was determined to be at 5-6 hours post-dosing. On the basis of the results, the author determined that 100mg/kg bw was a reasonable dose to use as the high dose in the present study and that observations on the day of dosing should be conducted 5-6 hours post-dosing..

TABLE 1: STUDY DESIGN

Test Group	Dose Level (mg/kg bw)	Number of Animals Examined					
		Neurobehavioural studies [b]		Necropsy		Neuropathology	
		M	F	M	F	M	F
Control [a]	0	10	10	6	6	5	5
Low	10	10	10	6	6	0	0
Mid	30	10	10	6	6	0	0
High	100	10	10	6	6	5	5

[a] Control animals received the vehicle at the same volume as the treated animals (10 mL/kg body weight)
[b] Motor activity was determined pretest (day -1), day 0 (6 hours after treatment), and at 7 and 14 days following treatment. FOB assessments were performed pretest, day 0 (6 hours after treatment), and at 7 and 14 days following treatment

4. **Test solution preparation and analysis:** Test solution was prepared on four occasions and used within 48 hours. The test material was ground into a fine powder then mixed with 0.5% sodium carboxymethylcellulose to form a suspension. Appropriate amounts of test substance were mixed with the vehicle such that animals could be dosed at a constant volume of 10ml/kg bw. Dosing solutions at concentrations of 0.1, 0.3 and 1.0% w/v were prepared for the 10, 30, or 100 mg/kg bw dose groups respectively. The suspension was administered with a rubber catheter attached to a syringe of appropriate size. During dosing the suspensions were stirred using a magnetic stirrer. Homogeneity and stability were tested using HPLC analysis. Samples at two dose levels were mixed for homogeneity and stability analysis (0.1 and 20 mg/mL).

Results -Homogeneity Analysis: Measured concentrations of acetamiprid ranged from 97 - 102% for the 0.1 mg/mL level, and from 94 - 102% for the 20mg/mL level when measured at the bottom, middle and top of the sample.

Stability Analysis: The test material was tested for stability by being held at room temperature or refrigerated for 2 days (similar to test conditions). The results of the stability analysis indicated that the test material was stable when held at either temperature for up to two days, with concentrations within ±4% of nominal concentrations.

Concentration Analysis: Measured concentrations of acetamiprid were 96.3 - 100% of nominal, indicating that the test substance was at expected concentrations in the dosing formulations.

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

5. **Statistics** - Food consumption and body weight gains were analysed on a weekly basis. Bartlett's test was used to test for variance between treatment groups. A one way ANOVA was then applied to the data. ANOVA was followed by a students t-test to assess any dose response relationships. For behavioural data including: rearing and activity counts, grip strength, hindlimb foot splay, body temperature, and activity data, an ANOVA followed by Williams' test for dose response was applied. When recorded observations suggested a possible treatment effect, the data was analysed using the Jonckheere-Terpstra test.

C. METHODS:

1. **Clinical Observations:** Animals were inspected daily for signs of toxicity and mortality.

2. **Neurobehavioural Studies:** The neurobehavioural evaluation consisted of an FOB and determination of motor and locomotor activity. All evaluations were performed by trained observers who did not know the identity of the dosed animals.

   a. Motor Activity Evaluation: All animals were evaluated for motor activity over a 60 minute test period (recorded in 2 minute blocks) on days 1 (6 hours following dosing), 7, and 14 in one of several randomly assigned automated activity monitors. The device measured duration and number of movements using infrared beams.

   b. FOB Evaluations: A functional observation battery (FOB) was carried out in all animals one day prior to treatment, on day 1, on day 7 and on day 14. Males and females were counterbalanced by gender and dose to minimize confounding variables. The following FOB parameters were evaluated:

OPEN FIELD OBSERVATIONS	HOME CAGE OBSERVATIONS
Level of activity	Posture
Rearing count	Palpebral closure
Convulsions, tremors, twitches	Gait abnormalities
Grooming	Vocalizations
Gait assessment	**MANIPULATIONS and MEASUREMENTS**
Arousal	Hindlimb grip strength
Presence or absence of Fecal boluses	Forelimb grip strength
Urination	Foot splay
HAND-HELD OBSERVATIONS	Righting reflex
Ease of removal from cage	Approach response
Reaction to handling	Touch response
Piloerection	Auditory response
Lacrimation	Tail pinch response
Salivation	Pupil response
Exophthalmos	Body temperature
Palpebral closure	Body weight

3. **Body weight:** Animals were weighed one week prior to dosing, on the day of dosing, and on days 7 and 14.

4. **Food consumption:** Food consumption was determined on a weekly basis by measuring the difference between the food remaining at the end of the week and the food provided at the beginning of the week.

5. **Sacrifice and Pathology:** At the end of the study all animals were sacrificed on schedule and tissue samples were taken. Tissues from 5 animals/sex from the control and high dose groups were subjected to neuropathological examination. Animals were anaesthetized with sodium pentobarbital, then perfused with heparinised solution. After perfusion, the brains were removed from the craniums and weighed. The skin was removed from the dorsal regions and the sciatic, tibial and sural nerves exposed. The brain was transected from the spinal cord above the first cervical spinal nerve and the olfactory bulbs removed. Rostral-caudal and left-right measurements of the brain were recorded. Following an overnight storage in fixative, tissues were prepared for paraffin wax sectioning. Brain, spinal cord, ganglia, dorsal and ventral root fibres were sectioned at 5-6 µm and stained with haematoxylin and eosin. Peripheral nerves were processed for epon/toluidine blue staining (sectioned at 2µm). Tissues examined are listed below:

Paraffin wax/H&E Sections	
Forebrain (3 cross-sections)	Spinal cord (cervical (C3-C6) and Lumbar (L1-L4)
Mid-brain (cross-section)	Gasserian ganglion
Cerebellum/Pons (cross-section)	Dorsal root ganglion/fibres (1 cervical and 1 lumbar each)
Medulla (cross-section)	Ventral root fibers (1 cervical and 1 lumbar) (longitudinal-section)
Epon/Toluidine Blue Sections	
Sural nerve (at knee and distal to the knee) (cross and longitudinal-section)	
Sciatic nerves (sciatic notch and mid-thigh) (cross and longitudinal-section)	
Tibial nerves (at knee and distal to the knee) (cross and longitudinal-section)	

II. RESULTS

A. Observations :

1. **Clinical signs of toxicity:** Clinical signs of toxicity were only observed in the high-dose animals. Males (3/10) were observed to have tremors (individual males had hunched posture or unsteady gait) which were evident only on the day of dosing. High-dose females (5/10) were also observed to have tremors on the day of dosing. One female had slight brown nasal staining from day 2 until the end of the study. The signs of body tremors in the high-dose animals were most evident at 7-8 hours post dosing (correlated with the estimated time of peak activity of the test compound) and were considered signs of neurotoxicity.

2. **Mortality** - There were no mortalities during the course of the study period.

B. **Body weight and weight gain:** Males in the high-dose group had significantly decreased body weight gain (86%) relative to the control animals over the first week of the study ($P<0.05$). The body weight gain was similar between groups during the second week of the study, however the total body weight gain for the high-dose males was significantly decreased relative to controls (88%, $P<0.05$). There were no effects on body weight or body weight gain seen in the females during the course of the study.

TABLE 2a: Mean Body Weight (g)*

STUDY DAY	DOSE LEVEL (mg/kg bw)			
	0	10	30	100
	Males			
0	201±14.7	205±11.7	201±15.2	199±12.7
7	278±15.7	283±14.2	281±20	265±16.2
14	322±18.5	322±15	322±24	305±19.5
	Females			
0	154±11.7	155±10.2	157±12.4	158±13
7	193±11.9	199±10.6	200±12.3	200±9.9
14	215±13.2	221±11.2	220±11.9	224±13.8

* Data obtained from page 36 of the study report.
* Significantly different ($p < 0.05$) from the control.

TABLE 2b: Mean Body Weight Gain (g)

STUDY Week	DOSE LEVEL (mg/kg bw)			
	0	10	30	100
	Males (% of control)			
0-1	77	78(101)	80(104)	66*(86)
1-2	43	39(91)	41(95)	41(95)
0-2	121	117(97)	121(100)	106*(88)
	Females (% of control)			
0-1	39	45(115)	43(110)	42(108)
1-2	22	21(95)	20(91)	24(109)
0-2	61	66(108)	63(103)	66(108)

* Data obtained from page 36 of the study report.
* Significantly different ($p < 0.05$) from the control.

C. <u>Food Consumption and Efficiency</u>: Similar to the effect on body weight gain, high-dose males had significantly reduced food consumption relative to controls during the first week of the study (90.5%; $P<0.05$). There were no effects on food consumption in females during the study. There were no effects on food efficiency in either male or female rats during the course of the study.

Table 3: Average Food Consumption (g/animal/week)

Study Week	DOSE LEVEL (mg/kg bw)			
	0	10	30	100
	Males			
-1	205	207	205	199
1	233±12.7	237±17.9	234±22.1	211±24.6*
2	231±8.4	231±17.1	235±20.5	223±21.5

Females				
-1	139	137	143	141
1	173±10.8	180±12.7	182±9.2	172±6.9
2	175±18.3	176±11	175±12.9	179±11.5

[a] Data obtained from page 37 of the study report.
* Significantly different ($p < 0.05$) from the control.

D. Motor Activity: The mean number of movements was not affected by treatment. The duration of movements was significantly decreased in mid- and high-dose males and in high-dose females on the day of dosing. A slight decrease in the duration of movements (not significantly different) persisted in mid- and high-dose males on days 7 and 14. The study author did not consider the day 7 and 14 decrease in activity among male animals to be treatment-related.

TABLE 4: Motor Activity [a]

Sex	Day	Dose (mg/kg bw)			
		0	10	30	100
Mean number of movements/2 minute sample time					
Male	baseline	9±4.7	8±4.2	8±4.8	8±6
	day1	2±0.7	4±4	1±1	2±1.9
	day 7	6±4.8	7±5.6	5±5.5	6±6.2
	day 4	7±6.5	5±3.1	8±3	6±5.6
Female	baseline	11±4.7	11±5.1	10±3.2	8±4.2
	day1	4±4	5±3	4±2.5	2±1.8
	day 7	17±5.3	18±5.5	12±7.9	13±8.4
	day 14	13±6.9	18±4.5	11±8.3	12±7.9
Mean Duration of Movements in seconds (% of Baseline)					
Male	baseline	544±303	512±193	633±211	588±235
	day1	256±83 (47)	235±153 (46)	**154±40* (24)**	**45±54* (8)**
	day 7	642±148 (118)	596±310 (116)	594±174 (94)	456±106 (78)
	day 14	593±148 (109)	599±260 (117)	511±177 (81)	495±170 (84)
Female	baseline	575±298	544±94	662±200	553±268
	day1	258±102 (45)	250±92 (46)	219±66 (33)	**33±25* (6)**
	day 7	586±329 (102)	655±280 (120)	654±320 (99)	586±265 (106)
	day 14	645±312 (112)	590±244 (108)	535±296 (81)	504±280 (91)

[a] Data obtained from pages 44 & 51 in the study report.
* Significantly different ($p < 0.05$) from the control.

E. Functional Observation Battery (FOB):

1. **FOB Evaluations:** On the day of dosing the high-dose males exhibited several treatment-related signs of neurotoxicity including marked tremors, difficulty in handling ($P<0.05$), walking on toes, dilated pupils ($P<0.05$), and coldness to the touch. There were no clear signs of neurotoxicity observed in males during the FOB evaluations on day 7 or 14.

   Females in the high dose displayed several signs of neurotoxicity on the first day of the observation period including tremors, chewing, coldness to the touch and dilated pupils. The incidence of gait

and/or posture abnormalities was increased among high-dose females, including the observation of walking on toes and hunched posture. There was no clear evidence of signs of neurotoxicity at the FOB evaluations on day 7 or 14.

TABLE 5a: Summary of FOB Findings (Number of animals showing signs) (N=10/group)

Males												
Observation	Day 1				Day 7				Day 14			
	0	10	30	100	0	10	30	100	0	10	30	100
Marked tremors	0	0	0	4	0	0	0	0	2	0	0	1
Difficult to handle	0	2	1	4*	1	0	1	0	0	0	0	0
Walking on toes	0	0	0	3*	0	0	0	1	0	0	0	1
Pupils dilated	0	0	0	3*	0	0	1	0	0	0	0	1
Cold to the touch	0	0	0	2	0	0	0	0	0	0	0	0
Females												
Observation	Day 1				Day 7				Day 14			
	0	10	30	100	0	10	30	100	0	10	50	100
Marked tremors	0	0	0	4	0	0	0	0	0	0	0	0
Walking on toes	0	4	2	5	1	6	1	3	2	4	3	3
Hunched	0	2	2	4*	0	0	0	0	0	0	0	0
Chewing	0	1	0	5*	0	0	0	0	0	0	0	0
Pupils dilated	0	0	0	6*	0	0	1	1	0	0	2	1
Cold to the touch	0	0	0	4*	0	0	0	0	0	0	0	0

Data obtained from pages 79-141 in the study report
* Significantly different ($p < 0.05$) from the control.

2. **FOB Measurements:** High-dose males and females had significantly reduced body temperature on the day of dosing ($P<0.05$). Forelimb grip strength was increased in females on the day of dosing. In addition, foot splay was decreased in high-dose males and females on the day of dosing. This effect in high-dose males was not statistically significant, but was considered treatment related by the study author and the reviewer.

Table 5b: FOB Measurements[a]

Sex	Day	Dose (mg/kg bw)			
		0	Low	Mid	High
		Forelimb Grip Strength (kg) (% of baseline)			
Male	baseline	0.77	0.75	0.81	0.82
	1	0.71 (92)	0.81 (108)	0.82 (101)	0.94* (115)
	7	0.82 (106)	0.99 (132)	0.86 (106)	0.97 (126)
	14	1.05 (136)	1.18 (157)	1.05 (130)	0.99 (121)

Female	baseline	0.8	0.75	0.74	0.68
	1	0.77 (96)	0.75 (100)	0.81 (109)	0.86 (126)
	7	0.88 (110)	0.86 (115)	0.89 (120)	0.94 (138)
	14	1.01 (126)	0.99 (132)	0.96 (130)	0.98 (144)
Mean Body Temperature					
Male	baseline	37.5	37.5	37.9	37.5
	1	37.1	37.3	37.1	**35.4***
	7	38	37.8	38.1	37.9
	14	37.8	37.6	38	38
Female	baseline	38.3	38.4	38.3	38.3
	1	37.4	37.5	37.4	**34.9***
	7	39	39.1	38.9	38.8
	14	39.1	39.1	38.9	38.8
Hindlimb Grip Strength (kg) (% of baseline)					
Male	baseline	0.59	0.63	0.64	0.61
	1	0.64 (108)	0.7 (111)	0.71 (111)	0.81 (133)
	7	0.81 (137)	0.88 (140)	0.86 (134)	0.85 (139)
	14	0.9 (153)	1.01 (160)	0.95 (148)	0.87 (143)
Female	baseline	0.68	0.67	0.72	0.67
	1	0.75 (110)	0.69 (103)	0.76 (106)	0.73 (109)
	7	0.8 (118)	0.73 (109)	0.84 (117)	0.78 (116)
	14	0.89 (131)	0.78 (116)	0.89 (124)	0.83 (124)
Foot Splay (cm) (% of baseline)					
Male	baseline	8.3	9.3	8.1	8.7
	1	7.9 (95)	9.2 (99)	7.5 (93)	6.7 (77)
	7	8.3 (100)	8.1 (87)	7.7 (95)	8 (92)
	14	8.6 (104)	9.6 (103)	9.1 (112)	8.6 (99)
Female	baseline	8.7	7.7	6.6	7.8
	1	8.6 (99)	8 (104)	7.2 (109)	**6.1* (78)**
	7	9 (103)	7.8 (101)	7.7 (117)	8.8 (113)
	14	9.5 (109)	9.1 (118)	8.9 (135)	8.1 (104)

* Data obtained from pages 46-49 in the study report.
* Significantly different ($p < 0.05$) from the control.
# in () are % of baseline

F. Sacrifice and Pathology:

1. Brain weight: There were no treatment-related effects on brain size or weight noted during the study.

2. Gross pathology: There were no treatment-related gross pathology measurements or observations recorded during the study.

3. Microscopic pathology: Microscopic findings included slight degeneration of axons of cervical and lumbar spinal cord, dorsal root fibres and ganglions, and trace axonal degeneration of mid-thigh sciatic nerve in some high-dose male and female rats. However, these signs of neurodegeneration were either found in control animals or were found in isolated animals without corresponding clinical signs of toxicity. As such, none of the neuropathology findings were deemed to be the result of acetamiprid treatment.

III. DISCUSSION

A. **Investigators' conclusions**: "A single acute dose of acetamiprid at dosages of 0, 10, 30 or 100 mg/kg was associated with effects principally at 100mg/kg.

"The acute dose of 100mg/kg was associated with decreased weight gains and decreased food consumption for males during the week following treatment. There was no similar effect among females.

"Behavioural changes were confined to the day of dosing. Six hours after an acute dose of acetamiprid a range of effects were observed particularly at 100mg/kg. Behavioural observations at 100mg/kg included observations of tremor, dilated pupils, increased urination (among males), altered patterns of gait and posture. There were clear effects on temperature which was reduced. Grip strength was increased and landing foot splay was decreased. The level of locomotor activity was reduced.

"At 30mg/kg, there was evidence of tremor among males (one animal showed continuous tremor); one female showed tail tremor. There was also an indication of increased urination among males. There was a statistically significant reduction of locomotor activity among males.

"At 10mg/kg, one female showed chewing behaviour. This singular occurrence in the absence of other changes was not considered clearly related to treatment as there was no evidence of chewing at the intermediate dosage.

"The main effect of treatment would appear to be on the central nervous system with the observation of tremors. There were some effects on the autonomic nervous system as indicated by observations of increased urination and increased pupil diameter/dilation. The change in grip strength and foot splay would appear to be enhanced performance and are suggestive of an increase in muscle tone. There were no apparent effects on sensory systems.

"There was no evidence of neuropathology.

"Based on these findings, the no observable effect level was considered to be 10mg/kg."

B. **Reviewer comments**: In an acute neurotoxicity study, acetamiprid was administered by gavage to 10 Crl:CD-BR rats/sex/group at doses of 0, 10, 30 or 100 mg/kg bw.

There were no treatment related mortalities during the study and 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.

Body weight gain was reduced in high-dose males in the first week of treatment, which resulted in an overall reduction in body weight gain in this group relative to controls ($P<0.05$). A corresponding significant reduction in food consumption in high-dose males was noted during the first week of the study. Body weight, body weight gain, food consumption and food efficiency were not affected in females.

Locomotor activity was reduced in mid- and high-dose males and high-dose females. The mean number of movements was not affected, however, the duration of movements was significantly decreased on the

day of dosing. A slight decrease in the duration of movements (not significantly different) 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.

High-dose males and females had significantly reduced body temperature on the day of dosing.

There were no treatment-related effects on brain size or weight and there was no evidence of neuropathology.

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.**

C. **Study deficiencies:** None noted.

PMRA Reviewer: Scott Hancock, **Date:** June 12, 2001
Secondary Reviewer: Gordon Cockell, **Date:** July 10, 2001

STUDY TYPE: Acute Neurotoxicity Range Finding- Rats OPPTS 870.6200; OECD 424.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical) 99.9%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Hughes, E.W. (1997) Acetamiprid Dose Range Finding Neurotoxicity to Rats by Acute Oral Administration. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number RNP/510/970145, October 28, 1997. MRID #44651841.Unpublished.

SPONSOR: Rhone-Poulenc Secteur Agro, France

EXECUTIVE SUMMARY: In an acute neurotoxicity range finding study (MRID #44651841), groups of fasted, male and female Crl:CD-BR rats (3/sex/dose), were given a single oral dose of Acetamiprid (99.9% pure) in 0.5% sodium carboxymethylcellulose by gavage, at doses of 10, 50, or 100 mg/kg bw and observed for 14 days.

All animals survived to study termination. A slight decrease in body weight gain was observed in females at 100 mg/kg bw. Body weight was unaffected in males as well as females in the 10 and 50 mg/kg bw dose groups. Clinical signs of toxicity included hind limb tremors in high-dose males, marked tremors in the limbs of high-dose females and dilatation of the pupils in high-dose females.

FOB evaluations revealed a number of treatment-related adverse behavioral observations, including reduced body temperature, hunched posture and constant grooming among high-dose males, moderate/marked body tremors, lower body temperature, hunched posture and dilated pupils in high-dose females. In addition, females treated at 50 mg/kg bw exhibited tail tremors and moderate body tremors. There were no clearly treatment related effects at 10 mg/kg bw, however, reduced body temperature was observed at all doses. Due to the small sample size, it is not possible to determine whether this observation is incidental or attributable to treatment with acetamiprid. The maximum signs of toxicity were observed during the functional observation battery (FOB) conducted 5 hours post-dosing.

The author concluded that 100mg/kg was a reasonable dose to use as the high dose in the acute neurotoxicity study, with a time to peak effect of approximately 5-6 hours following dosing.

This study is classified as supplemental and does not satisfy the guideline requirements for an acute neurotoxicity study (870.6200; OECD 424) in the rat. It was conducted for range finding purposes only.

COMPLIANCE: Signed and dated GLP, and Data Confidentiality statements were provided while a Quality Assurance statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** Acetamiprid

Description:	pale yellow powder
Lot/Batch #:	NFG-02
Purity:	99.9% a.i.
CAS #:	160430-64-8

2. **Vehicle and/or positive control:** 0.5% sodium carboxymethylcellulose

3 **Test animals:**

Species:	Rat	
Strain:	Crl:CD-BR	
Age/weight at dosing:	45 days/ males (190-241g), females (144-169g)	
Source:	Charles River Breeding Laboratories, Kent, England	
Housing:	individually in stainless steel mesh cages	
Diet:	SDS Rat No. 1 Maintenance diet *ad libitum*	
Water:	tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21 ± 2°C
	Humidity:	55 ± 9%
	Air changes:	not noted
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	10 days for males and 17 days for females	

B. STUDY DESIGN:

1. **In life dates** - **Start:** November 20, 1996 **End:** December 23, 1996

2. **Animal assignment:** Animals were assigned to the test groups noted in Table 1. Initially 3 males and 3 females were treated at 100 mg/kg bw at a concentration of 10 mg/mL. In order to determine a dose-response curve, a further 3 males and females were treated at 50 mg/kg bw at a concentration of 5 mg/mL. As there was some indication of a response to treatment, a further group of 3 animals per sex were treated at 10 mg/kg bw at a concentration of 1 mg/mL. A fourth group had been allocated to the study, however since there were no clear signs of reaction to treatment at 10 mg/kg, no further investigations were considered necessary.

Table 1: Study design

Test group	Dose (mg/kg bw)	# males	# females
Low	10	3	3
Mid	50	3	3
High	100	3	3

C. METHODS:

1. **Clinical Observations:** Animals were inspected daily for signs of toxicity and mortality.

2. **neurobehavioural Studies:** The neurobehavioural evaluation consisted of a Functional Observational Battery (FOB) performed on each animal before dosing and at ½, 2, and 5 hours post-dosing.

   a. Motor Activity Evaluation: Not performed

   b. FOB Evaluations: FOB evaluations were carried out in all animals one day prior to treatment, and ½, 2, and 5 hours post-dosing. Males and females were counterbalanced by gender and dose to minimize confounding variables. The following FOB parameters were evaluated:

OPEN FIELD OBSERVATIONS	HOME CAGE OBSERVATIONS
Level of activity	Posture
Rearing count	Palpebral closure
Convulsions, tremors, twitches	Gait abnormalities
Grooming	Vocalizations
Gait assessment	MANIPULATIONS and MEASUREMENTS
Arousal	Righting reflex
Presence or absence of fecal boluses	Approach response
Urination	Touch response
HAND-HELD OBSERVATIONS	Auditory response
Ease of removal from cage	Tail pinch response
Reaction to handling	Pupil response
Piloerection	Body temperature
Lacrimation/Salivation	Body weight
Vocalizations	
Exophthalmos	
Palpebral closure	

3. **Body weight:** Animals were weighed daily during the observation period.

4. **Food consumption:** Food consumption was not determined.

5. **Sacrifice and Pathology:** At the end of the study all animals were sacrificed on schedule and tissue samples were taken. However, no neuropathological examinations were performed.

II. RESULTS:

A. Observations:

1. **Clinical signs of toxicity and mortality:** There were no mortalities recorded during the study. The study author reported that there were no immediate post-dose signs of reaction to treatment. FOB evaluations (see below) revealed a number of adverse behavioral changes at 100 mg/kg, therefore

these animals were re-examined by placing them on a trolley approximately 6 hours after dosing. This revealed 2 males with tremors in the hindlimbs and 2 females with marked tremors in the limbs and noticeably dilated pupils.

2. **Body weight:** Body weight was not affected in males, however in females, initial body weight gain (day 0 to 1) was reduced at 100 mg/kg. Thereafter, body weight gain was comparable to controls. There was no effect on body weight at 10 or 50 mg/kg.

B. **Functional Observational Battery:** FOB evaluations revealed a number of observations that were possibly related to treatment with acetamiprid. Among males receiving 100 mg/kg, the predominant signs included constant grooming (½ hour evaluation), clearly reduced body temperature (2 and 5 hour evaluations), and hunched posture (5 hours). In addition, at 50 mg/kg, slight dilation of the pupils was observed prior to pupil reflex at ½ hour. Among females receiving 100 mg/kg, moderate/marked body tremors were observed at 5 hours, chewing and clearly lower body temperature were observed at 2 and 5 hours; hunched posture, enlarged area of hair loss, dilated pupils were observed at 5 hours. Constant grooming and slight dilation of the pupils prior to pupil reflex were observed among 50 mg/kg females (½ hour evaluation) as well as tail tremors at 2 hours and moderate body tremors at 5 hours. The author reported that a slight reduction in body temperature was observed at 10 and 50 mg/kg, however since there was no evidence of a dose-response curve, the change was not attributed to treatment. In the opinion of the reviewer, the observed reduction in body temperature at 10 and 50 may be related to treatment, but due to the small sample size (3 animals per group), a definitive conclusion regarding this observation cannot be reached.

Table 1: Body temperature observations recorded during FOB evaluations

Dose	Body Temperature (°C) Recorded During Study (change from pre-dose)							
	pre dose temperature		½ hour post dosing		2 hours post dosing		5 hours post dosing	
	Male	Female	Male	Female	Male	Female	Male	Female
10 mg/kg	38.1	38.3	38.1(-0.1)	38.4(0)	37(-1.1)	37.9(-0.4)	37.1(-1.0)	37.4(-0.9)
50 mg/kg	37.8	38.3	37.8(0)	38.4(0.1)	36.8(-1.0)	37.7(-0.6)	36.9(-0.9)	37.4(-0.9)
100 mg/kg	38.4	38.4	37.8(-0.6)	37.6(-0.8)	37(-1.4)	35.9(-2.5)	36.6(-1.8)	35.2(-3.2)

Data obtained from page 23 of the study report

C. Sacrifice and Pathology

1. **Gross Pathology:** There were no changes observed at necropsy that were attributed to treatment.

III. DISCUSSION

A. **Investigators' conclusions:** "Treatment of rats with acetamiprid resulted in a transient lower weight gain among females treated at 100 mg/kg and some behavioral changes. Changes included among males and females at 100 mg/kg: body tremors, hunched posture, and a clear lowering of body temperature. Pupil dilatation was also observed but only among females at 100 mg/kg. Body tremors were also

observed among females treated at 50 mg/kg. There were no clear effects of treatment at 10mg/kg.

"Based on these findings and clinical signs observations, the time of peak effect was established as between 5 to 6 hours.

"For the acute neurotoxicity study, a dosage of 100 mg/kg would appear to be a suitable highest dosage with post-dosing observations being performed at approximately 6 hours after dosing."

B. **Reviewer comments:** In a range-finding acute neurotoxicity study, 3 Crl:CD rats/sex/group were treated with acetamiprid by gavage at 10, 50 or 100 mg/kg bw and observed for 14 days.

All animals survived to study termination. A slight decrease in body weight gain was observed in females at 100 mg/kg bw. Body weight was unaffected in males as well as females in the 10 and 50 mg/kg bw dose groups. Clinical signs of toxicity included hind limb tremors in high-dose males, marked tremors in the limbs of high-dose females and dilatation of the pupils in high-dose females.

FOB evaluations revealed a number of treatment-related adverse behavioral observations, including reduced body temperature, hunched posture and constant grooming among high-dose males, moderate/marked body tremors, lower body temperature, hunched posture and dilated pupils in high-dose females. In addition, females treated at 50 mg/kg bw exhibited tail tremors and moderate body tremors. There were no clearly treatment related effects at 10 mg/kg bw, however, reduced body temperature was observed at all doses. Due to the small sample size, it is not possible to determine whether this observation is incidental or attributable to treatment with acetamiprid. The maximum signs of toxicity were observed during the functional observation battery (FOB) conducted 5 hours post-dosing.

The author concluded that 100mg/kg was a reasonable dose to use as the high dose in the acute neurotoxicity study, with a time to peak effect of approximately 5-6 hours following dosing.

C. **Study deficiencies:** No deficiencies were noted which would impact on the interpretation of the results.

DER #11

Acetamiprid: 90-Day Neurotoxicity Study in Rats
Rhone_Poulenc Secteur Agro. 1997. MRID No. 44651845

PMRA Reviewer: Scott Hancock, Date: June 6, 2001
Secondary Reviewer: Gordon Cockell, Date: July 18, 2001

STUDY TYPE: Subchronic Neurotoxicity - Rats OPPTS 870.6200.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical) 99.9%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Hughes, E.W. (1997) Acetamiprid Neurotoxicity to Rats by Dietary Administration for 13 Weeks. Huntingdon Life Sciences Ltd., Cambridgeshire, England. Laboratory report number RNP/511/971179, November 3, 1997. MRID #44651845. Unpublished.

SPONSOR: Rhone-Poulenc Secteur Agro, France

EXECUTIVE SUMMARY: 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.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1 **Test Material:** Acetamiprid

Description:	pale yellow powder
Lot/Batch #:	NFG-02
Purity:	99.9% a.i.
CAS #:	160430-64-8

2 **Test animals:**

Species:	Rat	
Strain:	Crl:CD-BR	
Age/weight at dosing:	47 days/ males (217-225g), females (172-176g)	
Source:	Charles River Breeding Laboratories, Kent, England	
Housing:	individually in stainless steel mesh cages	
Diet:	SDS Rat No. 1 Maintenance diet *ad libitum*	
Water:	tap water *ad libitum*	
Environmental conditions:	**Temperature:**	21.5 ± 2°C
	Humidity:	58 ± 13%
	Air changes:	not noted
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	12 days	

B. STUDY DESIGN:

1. **In life dates** - **Start:** December 16, 1996 **End:** March 21, 1997

2. **Animal assignment and treatment** - Animals were assigned to the test groups noted in Table 1 by a stratified randomization procedure to the test groups such that body weight means for each group were similar. Rats were fed diet containing the test substance *ad libitum* and the actual amount of test substance ingested was determined weekly. Survivors were sacrificed and neuropathology was performed.

TABLE 1: STUDY DESIGN

Test Group (ppm)	Dose Level (mg/kg) ♂/♀	Number of Animals					
		Initial		Neurobehavioral Studies		Neuropathology	
		M	F	M	F	M	F
Control (0)	0	10	10	10	10	5	5
100	7.4/8.5	10	10	10	10	0	0
200	14.8/16.3	10	10	10	10	0	0
800	59.7/67.6	10	10	10	10	0	0
1600	118/134	10	10	10	10	5	5

Motor activity and FOB assessments were conducted on pretest (day -1) and at week 4, 8, and 13 during the dosing regimen.

3. Diet preparation and analysis: A pre-mix was prepared every other week by grinding the test substance into the diet and mixing for 2 minutes in a Turbula mixer to achieve a homogeneous diet mixture. The pre-mix was diluted with further quantities of diet to generate each dose diet, and each diet preparation was further mixed for 5 minutes to achieve a homogeneous concentration in the diet. Homogeneity and stability were tested using HPLC analysis. Duplicate samples from the top, middle and bottom of the mixer were analyzed for the test material concentration and homogeneity prior to the initiation of the study. Samples were tested for stability at room temperature for 4, 8, and 15 days. During week 1 and week 11 of the study samples were analyzed for concentration.

Results - Homogeneity and Concentration Analysis: Measured concentrations of test substance ranged from 98 - 108% from all dose groups measured from samples taken during week 1 and week 11 of the study. In the test for homogeneity, samples ranged from 95.3 - 112% of the nominal concentration in samples taken from the bottom, middle, and top of the mixer. Coefficients of variation ranged from 1.89 - 6.86%. These data indicate that the test substance was distributed homogeneously and at the desired concentrations.

Stability Analysis: The compound was tested for stability after 15 days at room temperature. The compound was found to be at 97 - 103.5 % of nominal after 15 days at room temperature demonstrating that the compound was stable under the testing conditions.

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

4. Statistics: Food consumption and body weight gains were analysed on a weekly basis. Bartlett's test was used to test for variance between treatment groups. A one way ANOVA was then applied to the data. ANOVA was followed by a students t-test to assess any dose response relationships. For behavioral data including: rearing and activity counts, grip strength, hindlimb foot splay, body weight, body temperature, and activity data, an ANOVA followed by Williams' test for dose response was applied. When recorded observations suggested a possible treatment effect, the data was analysed using the Jonckheere-Terpstra test.

C. METHODS:

1. Clinical Observations: Animals were inspected daily for signs of toxicity and mortality. Animals were handled at each weighing and observed for abnormal behavior and appearance.

2. Neurobehavioral Studies: The neurobehavioural evaluation consisted of a functional observation battery (FOB) and determination of motor and locomotor activity.

a. Motor Activity Evaluation: All animals were evaluated for motor activity over a 60 minute test period (recorded in 6 separate 10 minute blocks) prior to initiation of dosing, and during weeks 4, 8 and 13, in an automated activity monitor. The device measured duration and number of movements using infrared beams.

b. FOB Evaluations: A functional observational battery (FOB) was carried out in all animals one day prior to treatment and during weeks 4, 8 and 13. Males and females were

counterbalanced by gender and dose to minimize confounding variables. Animals were scored in three environments; in their home cages, upon removal and while being handled, and in a standard open field arena. The following FOB parameters were evaluated:

HOME CAGE OBSERVATIONS	OPEN FIELD OBSERVATIONS
Posture	Defecation/Urination
Palpebral closure	Level of activity
Presence of convulsions, tremors or twitches	Presence of convulsions, tremors or twitches
Vocalizations	Arousal
HAND-HELD OBSERVATIONS	Rearing count
Ease of removal from cage	Grooming
Reaction to handling	Gait assessment
Presence of convulsions, tremors or twitches	**MANIPULATIONS**
Piloerection	Approach response
Vocalizations	Touch response
Salivation/ Lacrimation	Startle response
Palpebral closure	Tail pinch response
Body weight	Righting reflex
	Pupil response
	Grip strength (fore and hind limb)
	Landing foot splay
	Body temperature

3. **Body weight:** Animals were weighed prior to testing and weekly throughout the study period.

4. **Food consumption:** Food consumption was determined weekly by assessing the difference between the food offered at the start of the week and the amount of food remaining at the end of the week. Determinations of food utilization were derived from these weekly measures of body weight gain and food consumption.

5. **Sacrifice and Pathology:** At the end of the study all animals were sacrificed on schedule and tissue samples were taken. Tissues from 5 animals/sex from control and high dose groups were subjected to neuropathological examination. Animals were anaesthetized with sodium pentobarbital, then perfused with heparinised solution. After perfusion, the brains were removed from the craniums and weighed. The skin was removed from the dorsal regions and the sciatic, tibial and sural nerves exposed. The brain was transected from the spinal cord above the first cervical spinal nerve and the olfactory bulbs removed. Rostral-caudal and left-right measurements of the brain were recorded.

   Following an overnight storage in fixative, tissues were prepared for paraffin wax sectioning. Brain, spinal cord, ganglia, dorsal and ventral root fibres were sectioned at 5-6 µm and stained with haematoxylin and eosin. Peripheral nerves were processed for epon/toluidine blue staining (sectioned at 2µm). Tissues examined are listed below:

Tissues Examined Microscopically

Paraffin wax/H&E Sections	
Forebrain (3 cross-sections)	Spinal cord (cervical (C3-C6) and Lumbar (L1-L4)
Mid-brain (cross-section)	Gasserian ganglion
Cerebellum/Pons (cross-section)	Dorsal root ganglion/fibres (1 cervical and 1 lumbar each)
Medulla (cross-section)	Ventral root fibers (1 cervical and 1 lumbar) (longitudinal-section)
Epon/Toluidine Blue Sections	
Sciatic nerves (sciatic notch and mid-thigh) (cross and longitudinal-section)	Sural nerve (at knee and distal to the knee) (cross and longitudinal-section)
Tibial nerves (at knee and distal to the knee) (cross and longitudinal-section)	

II. RESULTS

A. Observations:

1. Clinical signs of toxicity: There were no clinical signs of toxicity recorded during routine monitoring of the animals at any time during the study.

2. Mortality: There were no mortalities during the course of the study.

B. Body weight and weight gain: Terminal body weights and body weight gains were significantly reduced relative to control values in both males and females at 800 and 1600 ppm. Slight reductions in body weight were observed in the 100 and 200 ppm dose group animals, however, these changes were not considered to be toxicologically significant.

TABLE 2: Average body weights and body weight gains during 90 days of treatment

Dose rate (ppm)	Body Weights (g)				Total Weight Gain	
	Week -1	Week 1	Week 8	Week 13	g	% of control
Male						
0	166	281	485	557	333	100
100	166	272	464	544	327	98
200	166	274	462	539	313	94
800	166	247	422	485	265*	80
1600	166	222	358	406	186*	56
Female						
0	145	200	294	327	152	100
100	145	198	288	317	144	95
200	145	201	289	315	139	91
800	144	181	252	272	100*	66*
1600	145	173	231	250	78*	51*

Data obtained from page 31 in the study report.
* Significantly different ($p < 0.01$) from the control.

C. Food Consumption/Efficiency: Similar to the effect seen on body weight and body weight gain, the highest two dose groups of both male and female rats were seen to have reduced food consumption compared to control values ($P<0.05$). There was a trend for animals to require more food to gain weight with an increase in dose of acetamiprid consumed (statistics were not performed on food efficiency). Females and males in the two highest dose groups required more food to gain the equivalent weight as the control animals. This observation was more pronounced among females, with high dose females requiring 159% more food than controls to gain an equivalent amount of body weight, as compared to 137% for high dose males.

Table 3: Food Consumption and Conversion Ratio

Dose rate (ppm)	Food Consumption (g)				Food Conversion Ratio *	% of Control
	Week 1	Week 8	Week 13	Mean		
Male						
0	199	211	205	215	8.4	100
100	200	204	203	209	8.3	99
200	199	204	208	209	8.7	104
800	160	191	186	192*	9.4	112
1600	114	171	172	165*	11.5	137
Female						
0	147	153	143	157	13.4	100
100	143	146	148	155	14	104
200	142	144	138	150	14	104
800	116	135	135	137*	17.9	134
1600	86	130	130	128*	21.3	159

Data obtained from pages 32-33 in the study report.
* Significantly different ($p<0.01$) from the control.
* food conversion is the amount of food consumed for every amount of body weight gain.

D. Motor Activity: There were no significant effects of treatment on motor activity recorded during this study.

TABLE 4: Motor Activity: (seconds) 10 animals/group

Sex	Day	Dose (ppm)				
		0	100	200	800	1600
Mean Duration of Movements (% of baseline)						
Male	baseline	254±99	251±132	206±80	234±75	246±80
	week 4	444±134 (175)	470±190 (187)	446±144 (217)	441±119 (217)	368±77 (150)
	week 8	630±229 (248)	671±384 (267)	522±194 (253)	541±120 (231)	466±192 (189)
	week 13	526±205 (207)	568±299 (226)	470±100 (228)	496±160 (212)	353±165 (143)
Female	baseline	351±155	352±145	339±133	338±102	382±133
	week 4	681±323 (194)	543±291 (154)	535±259 (158)	525±241 (155)	507±197 (133)
	week 8	671±252 (191)	639±272 (182)	478±267 (141)	542±300 (160)	514±199 (135)
	week 13	701±237 (200)	698±357 (198)	543±191 (160)	569±464 (168)	545±232 (163)

* Data obtained from page 46 in the study report.

E. Functional Observational Battery:

1. FOB Evaluations: During the FOB evaluations, there were a few noteworthy findings, however a time- and dose-dependent relationship to treatment could not be clearly established. There was a significant increase in vocalization in high dose female rats during the 4th week of the study. A similar increased incidence of vocalizing animals was recorded in high dose males during the 4th week. During the FOB evaluations at week 8 and 13, the incidence of vocalizing was similar in all groups, including controls.

A significant increase in the incidence of brown nasal staining was noted in high dose males and females during the 4th week. At week 8, all treated female groups had significantly increased incidence of brown nasal staining, however there was no increase in incidence or severity with the increasing dose. No difference between treated and control animals was apparent during the 13th week of the study. Due to the lack of a consistent dose- and time-relationship, these observations were not considered to be treatment-related by the study author or the reviewer.

Table 5a: FOB Evaluations:

Males															
Symptom	Week 4 (dose)					Week 8 (dose)					Week 13 (dose)				
	0	100	200	800	1600	0	100	200	800	1600	0	100	200	800	1600
brown nasal discharge	3	3	1	4	7*	4	3	5	5	4	3	5	3	8	5
vocalizing	1	1	1	0	4	5	2	2	1	2	6	3	2	2	5
Females															
Symptom	Week 4 (dose)					Week 8 (dose)					Week 13 (dose)				
	0	100	200	800	1600	0	100	200	800	1600	0	100	200	800	1600
brown nasal discharge	1	4	4	4	6*	2	7*	7*	8*	7*	4	1	5	4	4
vocalizing	1	3	2	2	6*	1	4	0	1	6	4	1	2	1	1

* Significantly different ($p < 0.01$) from the control.
Data obtained from pages 87-175 in the study report.

2. Limb Strength Evaluation: During the week 4 FOB, females at 800 and 1600 ppm had significantly reduced forelimb grip strength relative to concurrent controls. Females from all treated groups had significantly reduced forelimb grip strength recorded during the week 8 FOB, however the response was not dose-related. When compared to historical control data, the concurrent control value was at the high end of the normal range, and the observations among treated animals were within the normal range of variation.

Forelimb grip strength was reduced in high-dose males at week 13, and hind limb grip strength was reduced in males at 800 and 1600 ppm at week 13. The observed differences were well within the range observed in historical controls. Neither the study author nor the reviewer considered the limb strength observations to be related to treatment.

Table 5b: FOB Measurements

Sex	Week	Dose (ppm)				
		0	100	200	800	1600
Forelimb Grip Strength (Kg) (% of base line)						
Male	baseline	0.62	0.54	0.60	0.58	0.63
	4	1.28 (206)	1.16(215)	1.29(215)	1.18(203)	1.23(195)
	8	1.57 (253)	1.34 (248)	1.51 (252)	1.49(257)	1.40(222)
	13	1.74 (281)	1.38 (256)	1.53 (255)	1.53 (264)	1.36 (216*)
Female	baseline	0.65	0.61	0.60	0.61	0.63
	4	1.22 (188)	1.06(174)	1.15(192)	1.03 (169*)	1.09 (173*)
	8	1.41 (217)	1.04(170*)	1.29(215*)	1.13(185*)	1.19(189*)
	13	1.42(218)	1.06(174)	1.36(227)	1.23(202)	1.25(198)
Hindlimb Grip Strength (Kg) (% of base line)						
Male	baseline	0.68	0.64	0.67	0.61	0.69
	4	1.17 (172)	1.04 (163)	1.11(166)	1.00 (164)	1.07 (155)
	8	1.47(216)	1.19(186)	1.22(182)	1.18(193)	1.25(181)
	13	1.80(265)	1.44(225)	1.50(224)	1.46(239*)	1.40(203*)
Female	baseline	0.71	0.68	0.68	0.73	0.71
	4	1.07 (151)	1.10 (162)	1.05(154)	0.89 (122)	1.08(152)
	8	1.21(170)	1.35 (199)	1.14 (168)	1.05 (144)	1.16 (163)
	13	1.26(177)	1.40 (206)	1.15 (169)	1.18 (162)	1.22 (172)
Footsplay (cm) (% of base line)						
Male	baseline	7.8	6.9	7.3	6.7	7.4
	4	9.7 (124)	9.2 (133)	9.3(127)	9.7 (145)	10.4 (141)
	8	10.1 (129)	9.2 (133)	8.7 (119)	9.2 (137)	9.2 (124)
	13	10.5 (135)	8.7 (126)	9.1 (125)	9.1 (136)	9.8 (132)
Female	baseline	6.5	7.5	7.7	7.4	7.2
	4	7.5(115)	8.8 (117)	8.5 (110)	6.7 (91)	8.2 (114)
	8	7.5 (115)	9.2 (123)	8.8 (114)	7.1 (96)	8.4 (117)
	13	8.5 (131)	8.4 (112)	9.1 (118)	8.1 (124)	8.9 (124)

* Data obtained from pages 41-43 in the study report.
* Significantly different ($p < 0.01$) from the control.

Historical Control Data on Forelimb and Hindlimb Grip Strength *

Study Week	Males				Females			
	Forelimb		Hindlimb		Forelimb		Hindlimb	
	mean	range	mean	range	mean	range	mean	range
Predose	0.61	0.28-0.90	0.61	0.30-0.91	0.63	0.29-0.92	0.59	0.23-0.92
4	1.07	0.34-1.69	1	0.32-1.48	0.97	0.52-1.52	0.92	0.28-1.36
8	1.27	0.46-2.08	1.18	0.66-1.68	1.08	0.48-1.67	1	0.18-1.50
13	1.38	0.25-2.06	1.31	0.80-2.03	1.03	0.24-1.73	1.03	0.29-1.56

* Extracted from MRID 45130801 report supplement. Data from 9 studies, including data from this study.

F. Sacrifice and Pathology:

1. Brain weight: Brain weight and size measurements were not affected by treatment with acetamiprid.

2. **Microscopic pathology:** Trace axonal degeneration was observed in a number of neuronal tissues. The incidence of axonal degeneration was similar between control and high dose animals and as such was considered within the range of normal variation and not considered to be treatment related.

III. DISCUSSION

A. **Investigators' conclusions:** "Effects of treatment for 13 weeks with Acetamiprid were limited to the 800 and 1600 ppm dose groups. At 800 and 1600ppm treatment was associated with lower body weights and lower food consumption. There were no behavioural changes which were considered to be indicative of neurotoxicity nor were there any neuro-pathological findings which were attributed to treatment. The no observable effect level of the study was established at 200ppm based on body weight and food consumption reductions. No neurotoxic effects were observed following Acetamiprid treatment."

B. **Reviewer comments:** In a subchronic neurotoxicity study, 10 Crl:CD-BR rats/sex./group were treated with acetamiprid at dietary concentrations of 0, 100, 200, 800 or 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) for 90 days.

There were no mortalities or clinical signs of toxicity recorded during the study. Terminal body weights and body weight gains were significantly reduced relative to control values in males and females at 800 and 1600 ppm. Decreased food consumption and food efficiency were also observed among these animals.

There were no observations during the functional observational battery that were clearly related to treatment with acetamiprid. An increase in the incidence of vocalizing and brown nasal staining was observed among some treated groups, however these observations did not follow a temporal nor a dose-relationship and therefore were not deemed to be related to treatment. Some slight reductions in forelimb and/or hindlimb grip strength were observed in treated males and females, however the observations were well within the range of historical controls and there was no apparent dose-related trend. These slight changes were not considered to be related to treatment with acetamiprid. There was no evidence of neuropathology.

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).**

C. **Study deficiencies:** None noted.

DER #12

Acetamiprid: 21-Day Dermal Toxicity Study in Rats
Rhone Poulenc. 1997. MRID No. 44651844

Reviewer: Gordon Cockell, Date: February 2, 2001

STUDY TYPE: Repeat-dose Dermal Toxicity - Rabbit; OPPTS 870.3200 (rodent); OECD 410.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.9%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Trutter, J.A. (1997) 21-Day Dermal Toxicity Study in Rabbits with Acetamiprid. Covance Laboratories, Inc., Vienna, VA. Laboratory Study Identification Covance 6224-236, October 30, 1997. MRID 44651844. Unpublished

SPONSOR: Rhone Poulenc, Research Triangle Park, North Carolina

EXECUTIVE SUMMARY: In a repeat-dose dermal toxicity study (MRID 44651844), Acetamiprid (99.9% a.i.) was applied to the intact shaved skin of 5 New Zealand White rabbits/sex/dose at dose levels of 0, 100, 500 or 1000 mg/kg bw/day, 6 hours/day for 5 days/week over a 21-day period.

There were no compound related effects on mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology. **The NOAEL is 1000 mg/kg bw/day.**

This dermal toxicity study in the rabbit is acceptable and satisfies the guideline requirement for a repeat-dose dermal toxicity study (OPPTS 870.3200) ; OECD 410 in the rabbit.

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** Acetamiprid (NI-25)

Description:	white or pale yellowish white powder
Lot/Batch #:	NFG-02
Purity:	99.9 % a.i.
Compound Stability:	"Expiration date: September 30, 1999"
CAS #:	135410-20-7

2. **Vehicle:** Deionized water

3. **Test animals:**

Species:	Rabbit	
Strain:	New Zealand White	
Age/weight at study initiation:	3-4 months, males 2027-2535 g, females 2067-2671 g	
Source:	Covance Research Products, Inc., Denver, PA	
Housing:	Individual, in suspended, stainless steel, wire-mesh cages	
Diet:	PMI Certified Rabbit High Fiber #5325, *ad libitum*	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	17.6-22.1 °C
	Humidity:	41.9-57.4 %
	Air changes:	10 or greater per hour
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates - Start:** February 19, 1997 **End:** March 13, 1997

2. **Animal assignment**: Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Study design.

Test Group	Dose (mg/kg bw/d)	# Male	# Female
Control	0	5	5
Low	100	5	5
Mid	500	5	5
High	1000	5	5

3. **Dose selection rationale:** None provided, however acetamiprid was of low toxicity in the acute dermal toxicity study, where no mortality and no clinical signs of toxicity were noted at the limit dose of 2000 mg/kg bw.

4. **Preparation and treatment of animal skin:** Shortly before the first application and weekly thereafter, the fur of each test animal was clipped from the dorsal area of the trunk over an area of at least 10% of the body surface. Individual application amounts were adjusted after each weighing interval. The test substance was evenly dispersed on gauze patches, moistened with 2.5 mL of deionized water and then applied to the test site. An additional 2.5 mL of deionized water was used to rinse out the weighing container and this was added to the gauze patch. Dressings were held in place with non-irritating tape. The dressings were removed after 6-6.5 hours and the application areas were cleaned with deionized water and paper towels. Control animals were sham-treated using only deionized water.

5. **Statistics** - Body weights, body weight change, food consumption, clinical pathology data (except morphology gradings), and organ weight data of the treated groups were compared statistically to the data from the same sex of the control group. If variances of untransformed data were heterogeneous, a series of transformations was performed in an effort to achieve variance homogeneity. When the series of transformations was not successful in achieving variance homogeneity, analyses were performed on rank-transformed data. Group comparisons were performed at the 5% two-tailed probability level. The reviewer has no objections to the analyses used.

C. METHODS:

1. **Observations:** Animals were observed daily for signs of mortality, toxicity, and the presence of dermal irritation. Detailed physical examinations were conducted on days 1, 8, 15 and 22. The animals were examined for signs of local skin irritation on treatment days, immediately prior to administration of the test material and were evaluated using the Draize method.

2. **Body weight:** Animals were weighed prior to initiation of the study and at the beginning of each study week.

3. **Food consumption:** Food was provided and measured at least 3 times a week to obtain food consumption values for the periods of days 1-8, 8-15 and 15-21.

4. **Haematology & Clinical Chemistry:** Blood was collected on the day of necropsy from each animals by puncture of the medial ear artery. Animals were fasted overnight prior to collection of blood samples. The haematological and clinical chemistry parameters marked with an (X) in Tables (a) and (b), respectively, were examined.

a. Haematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for dermal toxicity studies based on Guideline 870.3200

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus		Total Cholesterol*
X	Potassium* (K)	X	Globulins
X	Sodium* (NA)	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	X	Total bilirubin
	Alkaline phosphatase (AP)*	X	Total serum protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio
X	Serum alanine amino-transferase (ALT/also SGPT)*		
X	Serum aspartate amino-transferase (AST/also SGOT)*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for dermal toxicity studies based on Guideline 870.3200

5. **Sacrifice and Pathology:** All animals were sacrificed on schedule and subjected to gross pathological examination. The CHECKED (X) tissues were collected from each animal and the preserved tissues from animals in the control and high-dose groups were subject to histological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*		Brain*+
	Salivary glands*		Heart*+		Peripheral nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*		Spleen*+		Eyes (optic nerve)*
	Jejunum*		Thymus*+		GLANDULAR
	Ileum*				Adrenal gland*+
	Cecum*		UROGENITAL		Lacrimal gland
	Colon*	XX	Kidneys*+		Mammary gland*
	Rectum*		Urinary bladder*		Parathyroid*
XX	Liver*+	XX	Testes*+		Thyroid*
n	Gall bladder*	XX	Epididymides*+		OTHER
	Pancreas*		Prostate*		Bone
	RESPIRATORY		Seminal vesicles*		Skeletal muscle
	Trachea*		Ovaries*+	X	Skin* (treated & untreated areas)
	Lung*		Uterus*+		All gross lesions and masses*
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for dermal toxicity studies based on Guideline 870.3200
+ Organ weights required.

II. RESULTS

A. Observations :

1. **Mortality** - No morality occurred during this study.

2. **Clinical signs of toxicity** - No clinical signs of toxicity were noted in any of the control or treated animals.

3. **Dermal Irritation** - Very slight erythema (barely perceptible) was recorded in one female at 500 mg/kg bw/day on the day after the first application.

B. Body weight and weight gain: Treatment did not affect body weight nor body weight gain. The author reported that the observed fluctuations were considered incidental, and represented the normal variability seen in New Zealand White rabbits, some of which is associated with excessive handling of the animals. The isolated observation that attained statistical significance was associated with reduced food consumption among high dose males during the first week of the study, also considered to be due to collaring and wrapping of the animals. The author's interpretation appears reasonable.

TABLE 2. Average body weights and body weight gains during 21 days of treatment

Dose (mg/kg bw/day)	Body Weights (g)				Total Weight Gain (Day 1-21)	
	Week -1	Week 1	Week 2	Week 3	g	% of control
Male						
0	2030±135	2514±164	2717±181	2734±166	397±61	100
100	2037±92	2569±64	2742±44	2777±67	485±160	122
500	1978±94	2410±114	2570±160	2603±159	394±75	99
1000	2006±126	2324±76*	2511±88	2540±61	327±71	82
Female						
0	2184±141	2492±121	2582±101	2611±105	218±161	100
100	2098±64	2388±147	2536±219	2580±167	345±119	158
500	2273±145	2478±144	2634±132	2685±118	268±95	123
1000	2179±233	2342±146	2459±122	2475±92	134±145	61

[a] Data obtained from pages (48-50) in the study report.
* Significantly different ($p < 0.05$) from the control.

C. Food consumption: Reduced food consumption was observed among high dose males during the first week of the study. This also resulted in lower total food consumption over the 21-day study period in high dose males. The author considered this finding to be incidental, and attributed this observation in part to collaring and wrapping of the animals.

D. Blood analyses:

1. Haematology - There were no differences between treated and control animals that could be attributed to treatment.

2. Clinical Chemistry - A very slight increase in mean sodium concentration was observed in high dose males. This finding is not considered to be related to treatment. No similar observation was present in females and no other gross or microscopic pathology was noted in the study.

G. Sacrifice and Pathology:

1. Organ weight - There were no treatment-related differences between treated and control animals in the organ weights or organ-to-body weight ratios that were recorded in this study.

2. Gross pathology - No abnormal lesions were noted upon gross pathological examination of treated and control animals.

3. Microscopic pathology - A similar number of spontaneous lesions and incidental findings were apparent in both treated and control animals. None of the changes were attributed to treatment with acetamiprid.

III. DISCUSSION

A. Investigators' conclusions: "Clinical observations, signs of dermal irritation, body weight and weight gain data, food consumption measurements, clinical pathology, organ weight values, necropsy findings and macroscopic pathology data provided no evidence of toxicity in this study.

"Notable gross dermal findings were limited to an isolated occurrence of very slight erythema in one 500 mg/kg/day female rabbit.

"No compound-related histomorphologic findings were observed in liver, kidney, or treated and untreated skin of animals from the 0 and 1000 mg/kg/day groups. A similar incidence and severity of commonly seen spontaneous lesions and incidental findings were noted in the control and treated animals.

"In conclusion, under the conditions of this study, dermal application of Acetamiprid at dosage levels of 100, 500 and 1000 mg/kg/day for at least 3 consecutive weeks to rabbits of both sexes did not cause systemic toxicity, dermal irritation, or histomorphologic lesions in any of the tissues examined; therefore, the NOEL is 1000 mg/kg/day."

@ B. Reviewer comments: The study was conducted properly and the author's conclusions are acceptable. No treatment-related changes were noted in any of the parameters investigated. The NOAEL is 1000 mg/kg bw/day.

C. Study deficiencies: None.

DER #13

Acetamiprid: 28-Day Feeding Study in Dogs
Nippon Soda. 1998. MRID No. 45245306

Reviewer: Gordon Cockell, Date June 6, 2001

STUDY TYPE: Subchronic Oral Toxicity, Dietary - Dog; OPPTS§870.3150 [§82-1]; OECD 409.

TEST MATERIAL (PURITY): Acetamiprid (NI-25 technical), 99.46%

SYNONYMS: (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine

CITATION: Auletta, C.S. (1998) A 4-week oral toxicity study of NI-25 in the dog via dietary administration. Bio/dynamics, Inc. East Millstone, NJ. Study no. 91-3726. February 20, 1998. MRID No. 45245306. Unpublished.

SPONSOR: Nippon Soda, Tokyo, Japan

EXECUTIVE SUMMARY: 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.

This subchronic toxicity study is classified as supplementary because it was performed for range-finding and purposes only. It does not satisfy the guideline requirement for a subchronic oral study (82-1); OECD 409 in the dog.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. A Quality Assurance statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test Material:** NI-25 (Acetamiprid)

Description:	pale yellow powder
Lot/Batch #:	NNI-02
Purity:	99.46 % a.i.
Compound Stability:	Stable for 2 months in the dark at 50°C
CAS #:	135410-20-7

2. **Test animals:**

Species:	Dog	
Strain:	Beagle	
Age/weight at study initiation:	Approximately six months, males 11.0 kg (9.8-12.6), females 9.0 kg (8.2-10.4)	
Source:	Marshall Farms, U.S.A., Inc.	
Housing:	Individual, in elevated metal grid cages. Animals were provided with exercise according to Animal Welfare Standards, following Bio/Dynamics Standard Operating Procedures	
Diet:	Standard laboratory diet (Purina Certified Canine Meal Diet #5007), 400 g/animal/day, available for 22 hours per day.	
Water:	Tap water, available *ad libitum*	
Environmental conditions:	**Temperature:**	16-26°C
	Humidity:	30-83%
	Air changes:	Not stated
	Photoperiod:	12 hour light/dark cycle (7 am - 7 pm via automatic timer)
Acclimation period:	Approximately 6 weeks	

B. STUDY DESIGN:

1. **In life dates** - **Start:** January 24, 1992 **End:** March 3, 1992

2. **Animal assignment**: Animals were assigned randomly to the test groups noted in Table 1.

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animal (mg/kg bw/day)	# Male	# Female
Control	0	0	2	2
Low/high	125 (Jan 24 - Feb 4) 3000 (Feb 5 - Mar 3)	4.1/4.8 42.5/46.2	2	2
Low	250	8.4/8.7	2	2
Mid	500	16.7/19.1	2	2
High	1000	28.0/35.8	2	2

3. **Diet preparation and analysis:** Appropriate amounts of test substance were mixed with the Certified Diets to achieve the desired concentrations. Fresh diets were prepared weekly. Homogeneity and stability analyses were conducted on diets from group 2 (125 ppm) and group 5 (1000 ppm) prior to initiation of dosing. Three samples were taken from the top, middle and bottom sections of each dietary batch for homogeneity analysis. The stability analysis was conducted on diets stored at room

temperature for 15 days. Concentration analysis of test diets was conducted weekly to ensure that the diets were prepared at their intended concentrations.

Results - Homogeneity Analysis: The homogeneity of the test diets ranged from -4% to +5% of the mean concentrations, for samples taken from the top, middle and bottom of the mixture.

Stability Analysis: NI-25 was found to be stable in test diets when stored at room temperature over a period of 15 days. After 15 days of storage at room temperature, concentrations ranged from 89.6% to 103% of nominal concentrations.

Concentration Analysis: The mean test material concentration in prepared diets were 96.1%, 101.5%, 99.0%, 100.2% and 100.1% of nominal concentrations for the 125, 250, 500, 1000 and 3000 ppm dose groups, respectively.

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

4. **Statistics** - No statistical analyses were performed because of the small number of animals in each group.

C. **METHODS:**

1. **Observations:** Animals were inspected at least twice daily for signs of toxicity and mortality. Detailed physical examinations were conducted weekly

2. **Body weight:** Animals were weighed twice pretest, weekly during the treatment period and at study termination after fasting.

3. **Food consumption and test article intake:** Food consumption for each animal was measured and recorded daily (seven days per week) and reported as weekly means. Nominal test article intake (mg/kg bw/day) values were calculated as time-weighted averages from the food consumption and body weight data.

4. **Haematology & Clinical Chemistry:** Blood was collected from all animals pretest and prior to study termination (after at least 28 days of treatment) for haematology and clinical analysis. Blood was obtained from unanesthetized animals via the jugular vein. Animals were fasted overnight prior to blood collection. The CHECKED (X) parameters were examined.

a. **Haematology**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Erythrocyte morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. **Clinical Chemistry**

	ELECTROLYTES
X	Calcium*
X	Chloride*
	Magnesium
X	Phosphorus*
X	Potassium*
X	Sodium*
	ENZYMES
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
X	Creatine phosphokinase
	Lactic acid dehydrogenase (LDH)
X	Serum alanine amino-transferase (also SGPT)*
X	Serum aspartate amino-transferase (also SGOT)*
X	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

	OTHER
X	Albumin*
X	Blood creatinine*
X	Blood urea nitrogen*
X	Total Cholesterol
X	Globulins
X	Glucose*
X	Total bilirubin
X	Total serum protein (TP)*
	Triglycerides
	Serum protein electrophoresis
X	A/G ratio
	Phospholipids

* Required for subchronic studies based on Subdivision F Guidelines

5. **Sacrifice and Pathology:** Gross postmortem examinations were conducted on all animals and the CHECKED (X) tissues were collected for possible histopathological examination. No microscopic examinations were conducted. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM
	Tongue
X	Salivary glands*
X	Esophagus*
X	Stomach*
X	Duodenum*
X	Jejunum*
X	Ileum*
X	Cecum*
X	Colon*
X	Rectum*
XX	Liver*+
X	Gall bladder*
X	Pancreas*
	RESPIRATORY
X	Trachea*
X	Lung*
	Nose
	Pharynx
	Larynx

	CARDIOVASC./HEMAT.
X	Aorta*
X	Heart*
X	Sternum with bone marrow*
X	Lymph nodes (mesenteric, submandibular)*
X	Spleen*
X	Thymus*
	UROGENITAL
XX	Kidneys*+
X	Urinary bladder*
XX	Testes*+
XX	Epididymides
	Prostate
	Seminal vesicle
X	Ovaries
	Vagina
X	Uterus*

	NEUROLOGIC
XX	Brain*
X	Periph. nerve (sciatic)*
X	Spinal cord (3 levels)^T
X	Pituitary*
X	Eyes (optic n.)^T
	GLANDULAR
XX	Adrenal gland*
	Lacrimal gland^T
X	Mammary gland^T
XX	Parathyroids*++
XX	Thyroids*++
	OTHER
X	Skeletal muscle
X	Skin
X	All gross lesions and masses*

* Required for subchronic studies based on Subdivision F Guidelines
\+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.
T = required only when toxicity or target organ

II. RESULTS

A. Observations:

1. **Clinical signs of toxicity and physical examinations** - Weekly physical examinations were generally unremarkable. The females in the 3000 ppm dose group appeared emaciated at study termination. This was consistent with the recorded weight loss in these two animals over the course of the study.

2. **Mortality** - All animals survived throughout the study.

B. **Body weight and weight gain:** Significant body weight losses were recorded among animals that received the highest dietary concentration (3000 ppm). A slight change was also observed at 1000 ppm. All four animals in this group lost weight during the first week of the study. Thereafter, the body weight gains in this group were lower than control animals. Body weight and body weight gain in the other groups were comparable to controls.

TABLE 2: Body weight and body weight gain over the 28-day treatment period

Dose rate (ppm)	Body Weights (g)						Total Weight Gain	
	Week -1	Week 1	Week 2	Week 3	Week 4	Week 5	kg	% of control
Male								
0	10.1	10.5	10.8	11.0	10.9	-	0.8	-
125/3000	10.7	11.4	11.7	10.7*	10.7*	9.7*	-1.8	-225
250	10.2	11.0	11.2	11.3	11.5	-	0.9	113
500	10.9	11.2	11.3	11.5	11.7	-	0.5	63
1000	11.5	11.8	11.9	11.9	12.0	-	0.2	25
Female								
0	9.2	9.6	9.9	10.1	10.5	-	0.9	-
125/3000	8.7	9.0	9.3	8.2*	7.9*	7.4*	-1.6	-178
250	8.3	9.3	9.2	9.7	9.8	-	1.0	111
500	8.7	9.1	9.1	9.5	9.8	-	0.8	89
1000	8.9	8.7	8.7	8.7	8.9	-	0.1	11

Data extracted from page 41-42 of the study report
* Dosing at 3000 ppm

C. Food consumption and compound intake:

1. **Food consumption** - A marked decrease in food consumption was observed upon initiation of dosing at 3000 ppm. Food consumption at other dose levels was comparable to controls, with the exception of one male at 1000 ppm that consumed slightly less food than controls.

2. **Compound consumption** - Mean compound consumption is shown in Table 3, below.

TABLE 3: Mean test article intake (mg/kg bw/day)

Dietary concentration (ppm)	0	125/3000	250	500	1000
Males	0	4.1/42.5	8.4	16.7	28.0
Females	0	4.8/46.2	8.7	19.1	35.8

D. Blood analyses:

1. **Haematology** - There were no changes in hematological parameters that could be attributed to treatment with acetamiprid.

2. **Clinical Chemistry** - There were no changes in clinical biochemistry parameters that could be attributed to treatment with acetamiprid.

E. Sacrifice and Pathology:

1. **Organ weight** - Slightly reduced absolute and relative (to brain) kidney and liver weights were observed among 3000 ppm animals. These organ weights relative to final body weight were similar to control values. No other notable differences in organ weights were observed. The above changes were considered to reflect the body weight changes noted in these animals rather than a direct effect of treatment with acetamiprid. The reviewer concurs with this interpretation.

2. **Gross pathology** - There were no macroscopic changes observed at necropsy that were attributed to treatment with acetamiprid.

III. DISCUSSION

A. Investigators' conclusions: "Based on the body weight losses seen in animals receiving a concentration of 3000 ppm and the absence of a definitive effect at 1000 ppm, the no observed effect level (NOEL) for dietary administration of NI-25 to dogs for four weeks under conditions of this study is 1000 ppm."

B. Reviewer comments: In a 4-week range finding toxicity study in Beagle dogs, acetamiprid was administered in the diet at nominal concentrations of 0, 125/3000, 250, 500 or 1000 ppm, equal to daily average intakes over the study period of 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.

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. The study author did not consider the effect on body weight gain at 1000 ppm to be a significant adverse effect of treatment, and as such established the NOAEL at that dose. The overall body weight gain in males and females at that dose was 25% of control in males and 11% of control in females. 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.

C. Study deficiencies: None.

DER #14

IM-1-4 (Metabolite of Acetamiprid): 90-Day Feeding Study in Rats
Nippon Soda. 1999. MRID No. 44988426

DATA EVALUATION RECORD

ACETAMIPRID
(IM-1-4)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY – RAT [OPPTS 870.3100a (82-1a)]
MRID 44988426

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-78C

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Date: APR 10 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725

ACETAMIPRID

Subchronic Oral Toxicity [OPPTS 870.3100 (§82-1a)]

EPA Reviewer: Joycelyn Stewart, Ph.D. Joycelyn Stewart, Date 5/23/2001
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity – Rat (OPPTS 870.3100 [§82-1a])

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: none

TEST MATERIAL: Acetamiprid (99.6% a.i.)

SYNONYMS: IM-1-4

CITATION: Ivett, J.L. (1999) 13-Week dietary subchronic toxicity study with IM-1-4 in rats. Covance Laboratories Inc., 9200 Leesburg Pike, Vienna, Virginia 22182-1699. Laboratory Study ID: 6840-102, February 1, 1999. MRID 44988426. Unpublished.

SPONSOR: Nippon Soda Co., Ltd., Regulatory Affairs, Product Development Department, Agro Product Division, Shin Ohtemachi Building 3rd floor, 2-1, 2-Chome, Ohtemachi, Chiyoda-ku, Tokyo 100, Japan

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 44988426), groups of Sprague-Dawley Crl:CD®BR rats (10 rats/sex/group) were administered 0, 200, 600, 1800, or 5400 ppm of IM-1-4 (Lot No. NK-97127; 99.6% a.i.) in the diet for at least 90 days. Time-weighted average doses were 0, 12.8, 36.5, 112.2, and 319.3 mg/kg/day, respectively, for males and 0, 15.6, 44.6, 135.6, and 345.7-565.3 mg/kg/day, respectively, for females. Overall time-weighted average doses for the 5400-ppm females could not be calculated because food consumption data for week 6 was lost due to a computer malfunction.

All animals survived to scheduled sacrifice and no treatment-related clinical signs of toxicity were observed in treated animals of either sex. No biologically significant effects on body weights, body weight gains, or food consumption were noted for the 200-, 600-, and 1800-ppm males and females. Body weights of the high-dose groups were significantly ($p \leq 0.05$) less than the controls beginning at week 2. For high-dose males and females, absolute body weights during the study were 82-87% and 88-91%, respectively, of the control group levels. Weekly body weight gains by the high-dose groups were significantly ($p \leq 0.05$) less than the controls for males during weeks 1-4 and 8 and for females only during week 1. Overall body weight gains by the high-dose males and females were 66% and 73% ($p \leq 0.05$ for both), respectively of the control group levels.

209

Males in the 5400-ppm group had significantly ($p \leq 0.05$) reduced weekly food consumption values throughout the study as compared with the controls resulting in overall food consumption that was 74% of the controls. High-dose females had significantly ($p \leq 0.05$; 74-84% of controls) lower food consumption as compared with that of the controls throughout the study with the exception of weeks 12 and 13. Food efficiencies by the 5400-ppm males and females for the first week of the study were 25% and 39%, respectively, of their control group values. Thereafter, food efficiencies by the high-dose groups were similar to the controls.

No treatment-related lesions were noted at gross necropsy and no dose-related or biologically significant effects were seen on hematology, clinical chemistry, urinalysis, organ weights, or ophthalmologic parameters.

Treatment-related microscopic lesions were limited to the spleen in the 1800-ppm males and the 5400-ppm males and females. For the control, 1800-, and 5400-ppm males, increased pigment in the spleen was observed in 0/10, 3/10, and 7/10, respectively, with mean severity ratings of 0.0, 0.4vc(minimal), and 1.4 (minimal to slight), respectively. For the control and 5400-ppm females increased pigment in the spleen was observed in 1/10 and 8/10, respectively, with mean severity ratings of 0.2 (minimal) and 1.6 (minimal to slight), respectively. This lesion was not seen in any animal from the other treated groups.

Therefore, the LOAEL for male rats is 1800 ppm (112.2 mg/kg/day) based on increased pigment in the spleen. The LOAEL for female rats is 5400 ppm (345.7-565.3 mg/kg/day) based on decreased body weight and body weight gains and increased pigment in the spleen. The NOAELs for males and females are 600 ppm (36.5 mg/kg/day) and 1800 ppm (135.6 mg/kg/day), respectively.

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

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, Flagging, and Good Laboratory Practice Compliance statements were provided.

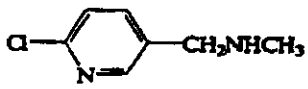
I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: IM-1-4

Description: white powder
CAS No.: not given
Lot No.: NK-97127
Purity: 99.6% a.i.
Contaminants: none given
Stability: stable on reanalysis

Structure:



2. Vehicle

Certified rodent diet (#8728C, Harlan Teklad, meal) was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: rat
Strain: Sprague-Dawley Crl:CD®BR
Age and weight at study initiation: approx. 6 weeks: males, 211-270 g; females, 154-192 g.
Source: Charles River Laboratories, Inc., Raleigh, NC
Housing: Animals were individually housed in stainless steel, hanging, wire-mesh cages.
Food: Certified rodent diet (#8728C, Harlan Teklad, meal) was available *ad libitum.*
Water: Tap water was available *ad libitum.*
Environmental conditions:
Temperature: 19.4-26.7°C
Humidity: 38.9-59.9%
Air changes: 10+/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 2 weeks

B. STUDY DESIGN

1. In life dates

Start: January 16, 1998 End: April 20-21, 1998

2. Animal assignment

Animal assignment and dose selection are listed in Table 1. Animals were assigned to test groups using a computerized weight-randomization program that produced homogeneity of variance and means.

TABLE 1. Study design					
Test group	Dietary Conc. (ppm)	Dose (mg/kg/day)		No. of animals	
		Males	Females	Males	Females
Control	0	0.0	0.0	10	10
Low	200	12.8	15.6	10	10
Mid	600	36.5	44.6	10	10
Mid -high	1800	112.2	135.6	10	10
High	5400	319.3	345.7-565.3[a]	10	10

Data taken from text table p. 16, MRID 44988426.
[a]Overall time-weighted average dose could not be calculated because food consumption data for week 6 was lost due to a computer malfunction.

3. Rationale for dose selection

A rationale for dose selection was not given.

4. Preparation and analysis of test diets

Test diets were prepared at least weekly during the study and stored at room temperature. For each dietary level, the required amount of test article was added to approximately 200 g of diet and this premix was mixed in a Waring blender for about 1-2 minutes. The premixes were added to the required amount of additional diet and mixed in a Hobart mixer for about 10 minutes. Concentration of the test article in each of the dietary levels was measured during test weeks 1, 5, 9, and 13. Homogeneity was analyzed in samples taken from the top, middle, and bottom of the low- and high-concentration diets prior to study initiation. Samples from the low- and high-concentration diets were analyzed for stability following storage at room temperature for 4, 7, and 10 days.

Results

Homogeneity analysis: Concentrations of the test article in samples from the top, middle, and bottom of the low- and high-concentration diets varied by <8%.

Concentration: Absence of test article was confirmed in the control diets. Concentrations of the test article in all diets were within 10% of nominal.

Stability: Following storage at room temperature, the low- and high-concentration diets were 98.7-99.7% and 97.2-98.6%, respectively, of their initial measured concentrations after 4 days and 89.5-90.5% and 91.5-92.7%, respectively, of their initial measured concentrations after 7 days. However, test article concentrations in the low- and high-concentration diets dropped to 77.5-79.6% and 87.4-90.5%, respectively, of their initial measured concentrations after 10 days of storage at room temperature.

Conclusion: These analyses confirm that the diets were homogeneously mixed and that the initial concentrations of the test article were acceptable.

5. Statistical analysis

Body weight, food consumption, clinical pathology, and organ weight data were analyzed by Analysis of Variance (ANOVA). If variances of untransformed data were heterogeneous, a rank transformation of the data was performed to achieve variance homogeneity. Data from the treated groups was compared statistically to the data from the same sex of the control group. Specific tests for group comparisons were not stated.

C. METHODS

1. Observations

Animals were observed once daily for clinical signs of toxicity and twice daily for mortality and moribundity. Detailed clinical examinations were conducted weekly on all animals.

2. Body weight

Body weights were recorded weekly during the study period.

3. Food consumption and food efficiency

Food consumption was measured weekly. Efficiency of food utilization was calculated as (mean weekly body weight gain/mean weekly food consumption) × 100. Compound consumption was calculated from body weight and food consumption data.

4. Ophthalmology

Indirect ophthalmic examinations were conducted on the eyes of all rats prior to initiation of treatment and during week 13 using 1% Mydriacyl® as the mydriatic agent.

5. Clinical chemistry

Blood was collected for hematology and clinical chemistry measurements from the orbital plexus of all rats prior to sacrifice using carbon dioxide anesthesia. Rats were fasted overnight prior to collection. The CHECKED (X) parameters were evaluated:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*	X	Blood cell morphology
	(Activated thromboplastin time)		Red cell distribution width
	(Clotting time)		
X	(Prothrombin time)		

*Required for subchronic studies based on OPPTS 870.3100 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
	Alkaline phosphatase (ALK)	X	Total serum protein*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Sorbitol dehydrogenase		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

*Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.

6. Urinalysis

Urine was collected during the overnight fast prior to blood collection. The CHECKED (X) parameters were measured:

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Urobilinogen
X	Protein		Reducing substances

Urinalysis is not required for subchronic studies.

7. Sacrifice and pathology

Following blood collection, all animals were weighed and sacrificed by an intraperitoneal injection of sodium pentobarbital and exsanguination. All rats were subjected to gross necropsy. The following tissues (X) were collected from all animals and preserved in 10% neutral buffered formalin. In addition, the (XX) tissues were weighed. All tissues from the control and high-dose animals and the liver, kidney, lung, and spleen from the animals in the lower dose groups were examined microscopically. All gross lesions from any animal were examined microscopically.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Oral tissues	X	Aorta*	XX	Brain*+
	Tongue	X	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary
X	Stomach*	X	Spleen*	X	Eyes (optic n.)
X	Duodenum*	X	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*		Auditory sebaceous gland
X	Rectum*	XX	Testes*+ (weighed with	X	Mammary gland*
XX	Liver*+		epididymides)	X	Parathyroids*
X	Pancreas*	X	Epididymides*	X	Thyroids*
		X	Prostate*		Coagulation glands
	RESPIRATORY		Seminal vesicle*		
X	Trachea*	X	Ovaries*		OTHER
X	Lung*		Oviducts	X	Bone*
	Nose (nasal turbinates)	X	Uterus*	X	Skeletal muscle*
	Pharynx		Cervix	X	Skin*
	Larynx		Vagina	X	All gross lesions and masses*

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in treated animals of either sex. Common findings in treated and control animals included alopecia, swellings on the paw or tail, and sores or scabs on the skin.

B. BODY WEIGHTS AND BODY WEIGHT GAINS

Selected mean body weights and body weight gains of males and females are listed in Table 2. Absolute body weights and body weight gains of the 200- and 600-ppm males and females were similar to those of the controls throughout the study. Absolute body

weights for the 1800-ppm males and females were slightly (n.s.) lower than those of the controls throughout the study due to slight decreases in body weight gains mainly during the first week of treatment. Body weights of the high-dose groups were significantly ($p \leq 0.05$) less than the controls beginning at week 2. For high-dose males and females, absolute body weights throughout the study ranged from 82-87% and 88-91%, respectively, of the control group levels. Weekly body weight gains by the high-dose groups were significantly ($p \leq 0.05$) less than the controls for males during weeks 1-4 and 8 and for females only during week 1. Overall body weight gains by the high-dose males and females were 66% and 73% ($p \leq 0.05$ for both), respectively of the control group levels.

TABLE 2: Selected body weights and body weight gains of male and female rats administered IM-1-4 in the diet for 13 weeks (g)

Week of study	0 ppm	200 ppm	600 ppm	1800 ppm	5400 ppm
Males					
1	240	244	240	240	247
2	292	295	294	287	254* (87)[a]
4	366	371	373	362	305* (83)
6	422	424	428	413	348* (82)
8	452	451	462	444	377* (83)
10	481	485	496	466	399* (83)
12	502	499	418	488	421* (84)
13	509	505	524	486	425* (83)
Wt. gain week 1	51	51	53	47	7* (14)
Wt. gain 1-6[b]	182	180	188	173	101 (55)
Wt. gain 1-13	277	268	292	250	183* (66)
Females					
1	169	169	176	167	169
2	192	187	198	185	174* (91)
4	224	221	237	219	200* (89)
6	246	244	262	236	216* (88)
8	259	262	275	243	229* (88)
10	270	273	288	258	240* (89)
12	274	280	296	265	245* (89)
13	274	280	300*	261	246* (90)
Wt. gain week 1	23	18	21	18	5* (22)
Wt. gain 1-6[b]	77	75	86	69	47 (61)
Wt. gain 1-13	106	112	126*	97	77* (73)

Data taken from Tables 3 and 4, pp. 61-62 and 63-64, respectively, MRID 44988426.
[a]Number in parentheses is percent of control; calculated by reviewer.
[b]Calculated by reviewer.
Significantly different from control: *$p \leq 0.05$.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption and food efficiency

Selected food consumption and food efficiency data are given in Table 3. It was noted that food consumption data for 5400-ppm females for week 6 were lost due to a computer malfunction; therefore, overall values for food consumption and food efficiency could not be calculated for this group.

Dose-related decreases in food consumption were observed in males at ≥600 ppm and in females at ≥1800 ppm. Food consumption by the 200-ppm males and females and by the 600-ppm females was slightly less than the control levels with statistical significance ($p \leq 0.05$) attained only for the 200-ppm males at week 8 (87% of controls). Males in the 600 ppm group had significantly ($p \leq 0.05$; 89-92% of controls) lower food consumption as compared with the controls during weeks 1-4, 7, and the combined interval of weeks 1-5. The 1800-ppm males had significantly ($p \leq 0.05$; 85-92% of controls) decreased food consumption for weeks 1-5 and 11-13 resulting in overall food consumption for the weeks 1-5, 7-13, and 1-13 intervals to be reduced to 90% ($p \leq 0.05$) of the control levels. Males in the 5400 ppm group had significantly ($p \leq 0.05$) reduced weekly food consumption values throughout the study as compared with the controls resulting in overall food consumption that was 74% of the controls. Weekly food consumption by the 1800-ppm females was only occasionally significantly less than that of the controls, however overall values for weeks 1-5 and 7-13 were 89-90% ($p \leq 0.05$) of the control levels. High-dose females had significantly ($p \leq 0.05$; 74-84% of controls) lower food consumption as compared with that of the controls throughout the study with the exception of weeks 12 and 13. Overall food consumption by the high-dose females could not be calculated because of the loss of data for week 6. However, overall food consumption by the high-dose females for weeks 1-5 and 7-13 was 81% and 80%, respectively, of the control group levels.

Food efficiency by the 200-, 600-, and 1800-ppm males and females was generally similar to the controls throughout the study. However, food efficiencies by the 5400-ppm males and females for the first week of the study were 25% and 39%, respectively, of their control group values. Reduced food efficiency during the first week resulted in lower overall values for the week 1-5 interval for the high-dose groups as compared with the controls. Thereafter, food efficiencies by the high-dose groups were similar to the controls.

TABLE 3: Selected food consumption and food efficiency of male and female rats administered IM-1-4 in the diet for 13 weeks

Sex/Interval	0 ppm	200 ppm	600 ppm	1800 ppm	5400 ppm
Food Consumption (g)					
Males					
Week 1-5	986	938	893* (91)[a]	886* (90)	680* (69)
Week 7-13	1296	1219	1238	1166* (90)	1036* (80)
Week 1-13	2448	2322	2311	2204* (90)	1813* (74)
Females					
Week 1-5	704	645	668	628* (89)	569[b] (81)
Week 7-13	927	915	912	836* (90)	740* (80)
Week 1-13	1776	1694	1695	1599	-
Food Efficiency (%)					
Males					
Week 1-5	18.6	19.3	21.1	19.7	14.5
Week 8-13	4.9	5.9	6.6	4.1	6.0
Week 1-13	11.0	11.4	12.6	11.3	9.6
Females					
Week 1-5	11.1	10.5	12.0	10.2	9.3
Week 8-13	2.0	2.3	3.4	1.7	2.6
Week 1-13	5.8	5.9	6.6	5.5	-

Data taken from Tables 5 and 6, pp. 65-66 and 67-68, respectively, MRID 44988426.
[a]Number in parentheses is percent of control; calculated by reviewer.
[b]Data not analyzed statistically because too few values available.
Significantly different from control: *$p \leq 0.05$.

2. Compound intake

Time-weighted average doses are given in Table 1. For males in the 200-, 600-, 1800-, and 5400-ppm groups, weekly compound consumption ranged from 9.9-19.5, 29.1-56.5, 83.4-167.4, and 264.7-405.7 mg/kg/day, respectively. Overall time-weighted average doses for males were 12.8, 36.5, 112.2, and 319.3 mg/kg/day, respectively. For females in the 200-, 600-, 1800-, and 5400-ppm groups, weekly compound consumption ranged from 12.9-21.2, 37.1-59.6, 110.3-179.2, and 345.7-565.3 mg/kg/day, respectively. Overall time-weighted average doses for females in the 200-, 600-, and 1800-ppm groups were 15.6, 44.6, and 135.6 mg/kg/day, respectively, but could not be calculated for the 5400-ppm group because of the lost data for food consumption.

D. OPHTHALMOLOGY

No treatment-related ophthalmologic lesions were observed in any animal. Unilateral retinal linear atrophy was seen in one 200-ppm male.

E. CLINICAL CHEMISTRY

No differences in any hematological parameter were noted between the treated and control groups of either sex. For the high-dose males the mean globulin value was significantly ($p \leq 0.05$) less and, consequently, the albumin/globulin ratio was significantly ($p \leq 0.05$) increased as compared with the controls. No other differences in any clinical chemistry parameters were noted.

F. URINALYSIS

No treatment-related differences were observed in urinalysis parameters between the treated and control rats of either sex.

G. SACRIFICE AND PATHOLOGY

1. Gross pathology

No treatment-related lesions were noted at necropsy.

2. Organ weights

Terminal body weights of the 5400-ppm males and females were significantly ($p \leq 0.05$) less than the controls. For the high-dose males, absolute liver weights were significantly ($p \leq 0.05$) decreased and relative (to body weight) brain, testis, and kidney weights were significantly ($p \leq 0.05$) increased as compared with the control. For the high-dose females absolute brain weights were significantly ($p \leq 0.05$) less than that of the controls. Terminal body weights of the 600-ppm females were significantly ($p \leq 0.05$) greater that the controls resulting in decreased relative brain, kidney, and adrenal weights for this group.

3. Microscopic pathology

Treatment-related microscopic lesions were limited to the spleen in the 1800-ppm males and the 5400-ppm males and females. Severity of lesions were graded on a scale of 1-5 for minimal, slight, moderate, marked, and severe, respectively. For the control, 1800-, and 5400-ppm males, increased pigment in the spleen was observed in 0/10, 3/10, and 7/10, respectively, with mean severity ratings of 0.0, 0.4, and 1.4, respectively. For the control and 5400-ppm females increased pigment in the spleen was observed in 1/10 and 8/10, respectively, with mean severity ratings of 0.2 and 1.6, respectively. This lesion was not seen in any animal from the other treated groups.

III. DISCUSSION

A. DISCUSSION

Treatment with the test article did not result in mortalities or cause clinical signs of toxicity in male or female rats. Dose-related decreases in food consumption resulted in statistically significant decreases in body weight only at the highest dose level. Significant decreases in food consumption by the 600-ppm males and the 1800-ppm males and females without corresponding effects on body weights were not considered biologically significant. The reductions in food consumption were most likely due to a lack of palatability.

At terminal sacrifice, differences in absolute and/or relative organ weights were considered a result of differences in final body weights of the treated groups as compared with the controls. The slight decrease in the globulin level for the high-dose males may have been related to the lower food consumption and body weight gains by these animals.

Increased pigment in the spleen was the main effect of test article administration. Both the incidence and severity were increased for males in the two highest dose groups and for females at the highest dose. However, changes in hematology parameters indicative of increased red cell turnover were not observed. Therefore, the biological significance of this microscopic finding is unknown.

Therefore, the LOAEL for male rats is 1800 ppm (112.2 mg/kg/day) based on increased pigment in the spleen. The LOAEL for female rats is 5400 ppm (345.7-565.3 mg/kg/day) based on decreased body weight and body weight gains and increased pigment in the spleen. The NOAELs for males and females are 600 ppm (36.5 mg/kg/day) and 1800 ppm (135.6 mg/kg/day), respectively.

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

B. STUDY DEFICIENCIES

No deficiencies were noted in the conduct of this study.

DER #15

IM-0 (Metabolite of Acetamiprid): 90-Day Feeding Study in Rats
Nippon Soda. 1997. MRID No. 44988427

DATA EVALUATION RECORD

ACETAMIPRID
(IM-0)

STUDY TYPE: SUBCHRONIC ORAL TOXICITY – RAT [OPPTS 870.3100a (§82-1a)]
MRID 44988427

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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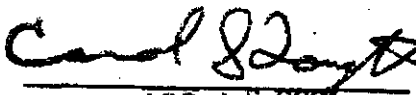
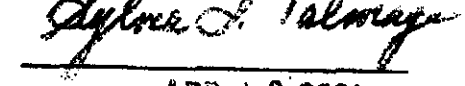
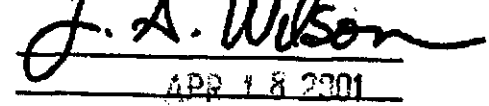
Task Order No. 01-78B

Primary Reviewer: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	 APR 18 2001
Secondary Reviewers: Sylvia S. Talmage, Ph.D., D.A.B.T.	Signature: Date:	 APR 18 2001
Robert H. Ross, M.S., Group Leader	Signature: Date:	 APR 18 2001
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	 APR 18 2001

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

ACETAMIPRID **Subchronic Oral Toxicity (OPPTS 870.3100 [82-1a])**

EPA Reviewer:Joycelyn E. Stewart, Ph.D. _____, Date 5/24/2001
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: S. Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity – Rat [OPPTS 870.3100 (§82-1a)]

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: none

TEST MATERIAL: Acetamiprid (98.94% a.i.)

SYNONYMS: IM-0; CPA; (6-chloro-3-pyridyl) methanol

CITATION: Nukui, T. and Ikeyama, S. (1997) IM-0 - Thirteen-week dietary subchronic toxicity study in rats. Toxicology Laboratory, Odawara Research Center, Nippon Soda Co., Ltd., 345 Takada, Odawara, Kanagawa, Japan 250-02. Laboratory Project ID: G-0889, November 28, 1997. MRID 44988427. Unpublished.

SPONSOR: Nippon Soda Co., Ltd., 2-2-1 Ohtemachi, Chiyodaku, Tokyo, Japan 100

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 44988427), groups of Crj:CD™(SD) rats (10 rats/sex/group) were administered 0-, 160-, 800-, 4000-, or 20,000-ppm of IM-0 (Lot No. NK-3266; 98.94% a.i.) in the diet for at least 90 days. Time-weighted average doses were 0, 9.9, 48.9, 250.1, and 1246.6 mg/kg/day, respectively, for males and 0, 11.1, 55.9, 275.9, and 1173.7 mg/kg/day, respectively, for females.

All animals survived to scheduled sacrifice and no treatment-related clinical signs of toxicity were observed in treated animals of either sex.

No dose- or treatment-related effects on body weights, body weight gains, food consumption, or food efficiencies were noted for the 160-, 800-, and 4000-ppm males and females. Body weights and body weight gains of the 20,000-ppm males and females were significantly ($p \leq 0.01$) less than the controls beginning at week 1. For high-dose males and females, absolute body weights were 77-80% and 76-83%, respectively, of the control group levels. Body weight gains by high-dose males and females were 29% and 9%, respectively, of the control group levels during the first week of the study and 67% and 57% ($p \leq 0.05$ for both), respectively, of the control group levels overall.

Food consumption by the 20,000-ppm groups was significantly ($p \leq 0.01$) less than the controls during weeks 1-4, 6, 9, and 13 for males and throughout the study for females. Food

consumption during week 1 for the males and females was 59% and 67%, respectively, of the control group levels. Thereafter, food consumption for the high-dose males ranged from 68% to 87% of the control values. However, food consumption for the high-dose females varied from 55% to 76% of the control values. Food efficiencies by the high-dose males and females for the first week of the study were 50% and 9%, respectively, of their control group values ($p \leq 0.01$). Thereafter, food efficiencies by the high-dose groups were similar to the controls with the exception of males at week 10 ($p \leq 0.05$; 63% of control).

No treatment-related lesions were noted at gross necropsy and no dose-related or biologically significant effects were seen on hematology, clinical chemistry, urinalysis, or ophthalmologic parameters. Differences in absolute and/or relative organ weights for the 20,000-ppm males and females as compared with the controls were attributed to significantly ($p \leq 0.01$) lower final body weights of the treated animals.

Treatment-related microscopic lesions were limited to an increased incidence ($p \leq 0.01$) of eosinophilic intranuclear inclusions in the proximal tubular epithelium of the kidney in the 4000-ppm males and the 20,000-ppm males and females. Severity of the lesion was rated on a scale of 1-3 designated slight, moderate, or marked, respectively. The incidence (severity) of the inclusions for the control, 4000-, and 20,000-ppm males was 0/10 (0), 7/10 (1.0), and 10/10 (2.7), respectively, and for the control and 20,000-ppm females was 0/10 (0) and 9/10 (1.8), respectively. This lesion was not observed in the other treated groups.

Therefore, the LOAEL for male rats is 4000 ppm (250.1 mg/kg/day) based on an increased incidence and severity of eosinophilic intranuclear inclusions in the proximal tubular epithelium of the kidney. The LOAEL for female rats is 20,000 ppm (1173.7 mg/kg/day) based on decreased body weights, body weight gains, food consumption, and food efficiency and an increased incidence of eosinophilic inclusions in the kidney. The NOAELs for males and females are 800 ppm (48.9 mg/kg/day) and 4000 ppm (275.9 mg/kg/day), respectively.

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

COMPLIANCE: Signed and dated Quality Assurance, Data Confidentiality, Flagging, and Good Laboratory Practice Compliance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound: IM-0

   Description: pale yellow crystal
   CAS No.: not given
   Lot No.: NK-3266
   Purity: 98.94% a.i.

Contaminants: none given
Stability: stable for 12 months at 0-5 °C
Structure:

2. Vehicle

Powdered basal diet (MF, Oriental Yeast Co., Ltd., Tokyo) was used as the vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: rat
Strain: Crj:CD™(SD)
Age and weight at study initiation: approx. 5 weeks: males, 180.5-207.3 g; females, 137.2-163.9 g.
Source: Charles River Japan, Inc., Kanagawa, Japan
Housing: Animals were individually housed in stainless steel, hanging, wire-mesh cages.
Food: Basal diet (Oriental Yeast Co., Ltd.) was available *ad libitum.*
Water: Tap water was available *ad libitum.*
Environmental conditions:
Temperature: 22.4±0.6 °C
Humidity: 60.7±1.8%
Air changes: 10-20/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 1 week

B. STUDY DESIGN

1. In life dates

Start: July 13, 1993 End: October 14, 1993

2. Animal assignment

Animal assignment and dose selection are listed in Table 1. Animals were assigned to test groups using a computerized randomization procedure that insured that the body weight means of each group were comparable.

TABLE 1. Study design					
Test group	Dietary Conc. (ppm)	Dose (mg/kg/day)		No. of animals	
		Males	Females	Males	Females
Control	0	0	0	10	10
Low	160	9.9	11.1	10	10
Mid	800	48.9	55.9	10	10
Mid -high	4000	250.1	275.9	10	10
High	20,000	1246.6	1173.7	10	10

Data taken from text table p. 25 and Text Table II, p. 31, MRID 44988427.

3. Rationale for dose selection

Doses were selected on the basis of an acute oral toxicity study in rats. In this study, LD_{50}'s of 1842 and 1483 mg/kg were identified for males and females, respectively.

4. Preparation and analysis of test diets

Test diets were prepared three times during the study at approximately one-month intervals and stored in a freezer until use. For each dietary level, the appropriate amount of test article was added to basal diet and mixed using a mixer for 7 minutes. Each prepared diet was transferred to different plastic bags and manually shaken to assure homogeneity. Concentration and homogeneity of the dietary mixtures was measured in samples taken from the top, middle, and bottom of each preparation. Stability of the test article in the diet was measured in sample preparations containing 92 ppm (stored at room temperature for 4 days) or 100 ppm (stored frozen for up to 35 days).

Results

Homogeneity analysis: Concentrations of the test article in samples from the top, middle, and bottom of one 160-ppm preparation varied by 16%. Concentrations in all remaining dietary levels and preparations varied by <10%.

Concentration: Absence of test article was confirmed in the control diets. Mean concentrations of the test article in all diets were within 6% of nominal.

Stability: Following storage at room temperature for 4 days, the test article concentration in the 92 ppm sample diet was 95.7% of the initial measured concentration. Following frozen storage for up to 35 days, the test article concentrations in the 100 ppm sample diet were within 8% of the initial measured concentration.

Conclusion: These analyses confirm that the diets were homogeneously mixed and that the initial concentrations of the test article were acceptable.

5. Statistical analysis

Ophthalmological, semi-quantitative urinalysis, and macroscopic and microscopic observational data were analyzed by the Chi-square test. Body weight, food consumption, hematology, biochemistry, quantitative urinalysis, and organ weight data were analyzed for homogeneity of variance by Bartlett's test. If the variances were equal, a one-way Analysis of Variance (ANOVA) was used to determine significance. For unequal variances, the Kruskal-Wallis test was used to determine significance. Means were compared by either Dunnett's or Scheffe's test.

C. METHODS

1. Observations

Animals were observed once daily for clinical signs of toxicity, mortality, and moribundity. Detailed physical examinations were conducted weekly on all animals.

2. Body weight

Body weights were recorded weekly during the study period.

3. Food consumption and food efficiency

Food consumption was measured weekly. Food efficiency was calculated as (body weight gain/food consumption) × 100. Compound consumption was calculated from body weight and food consumption data and nominal dietary concentrations.

4. Ophthalmology

Indirect ophthalmic examinations were conducted on the eyes of all rats in the control and high-dose groups at study initiation and during week 12 using Mydrin™-P as the mydriatic agent.

5. Clinical chemistry

Blood was collected for hematology and clinical chemistry measurements from the carotid artery of all rats prior to sacrifice using pentobarbital anesthesia. Rats were fasted for at least 16 hours prior to collection. Blood smears were made and examined for differential leukocyte counts. The CHECKED (X) parameters were evaluated:

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*		Blood cell morphology
X	(Activated thromboplastin time)		Red cell distribution width
X	(Fibrinogen concentration)		
X	(Prothrombin time)		

*Required for subchronic studies based on OPPTS 870.3100 Guidelines.

b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Albumin/globulin ratio
	Magnesium	X	Blood creatinine*
X	Phosphorus*	X	Blood urea nitrogen*
X	Potassium*	X	Total Cholesterol
X	Sodium*	X	Globulins
		X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein*
X	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Sorbitol dehydrogenase		
X	Alanine aminotransferase (also SGPT)*		
X	Aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.

6. Urinalysis

Urine was collected from all rats over a 24-hour fasting period during week 13. Rats were placed in metabolism cages during collection and water consumption was measured. The CHECKED (X) parameters were measured:

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)	X	Urobilinogen
X	Protein		Reducing substances

Urinalysis is not required for subchronic studies.

7. Sacrifice and pathology

All animals were weighed and sacrificed by exsanguination from the carotid artery while under pentobarbital sodium anesthesia. All rats were subjected to gross necropsy. The following tissues (X) were collected from all animals and preserved in 10% phosphate-buffered neutral formalin. In addition, the (XX) tissues were weighed. All tissues from the control and high-dose animals and the liver, kidney, lung, and target organs from the animals in the lower dose groups were examined microscopically. In addition, all gross lesions from any animal were examined microscopically. In addition, the kidneys from one control male and one high-dose male were examined by electron microscopy.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Oral tissues	X	Aorta*	XX	Brain*+
	Tongue	X	Heart*	X	Periph. nerve*
X	Salivary glands*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Esophagus*	X	Lymph nodes*	X	Pituitary
X	Stomach*	XX	Spleen*	X	Eyes (optic n.)
X	Duodenum*	XX	Thymus*		
X	Jejunum*				GLANDULAR
X	Ileum*		UROGENITAL	XX	Adrenal gland*
X	Cecum*	XX	Kidneys*+	X	Lacrimal gland
X	Colon*	X	Urinary bladder*	X	Harderian gland
X	Rectum*	XX	Testes*+	X	Mammary gland*
XX	Liver*+	X	Epididymides*	X	Parathyroids*
X	Pancreas*	X	Prostate*	X	Thyroids*
		X	Seminal vesicle*		Coagulation glands
	RESPIRATORY	XX	Ovaries*		
X	Trachea*	X	Oviducts		OTHER
XX	Lung*	X	Uterus*	X	Bone*
	Nose (nasal turbinates)		Cervix	X	Skeletal muscle*
	Pharynx	X	Vagina	X	Skin*
	Larynx			X	All gross lesions and masses*

* Required for subchronic toxicity studies based on OPPTS 870.3100 Guidelines.
+ Organ weight required in subchronic and chronic studies.

II. RESULTS

A. CLINICAL OBSERVATIONS AND MORTALITY

All animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in treated animals of either sex. Common findings in treated and control animals included alopecia and abnormal teeth.

B. BODY WEIGHTS AND BODY WEIGHT GAINS

Selected mean body weights and body weight gains of males and females are listed in Table 2. Absolute body weights and body weight gains of the 160-, 800-, and 4000-ppm

males and females were similar to those of the controls throughout the study with two exceptions. Cumulative weight gains were significantly ($p \leq 0.05$) less than the controls for the 160-ppm females at week 9 and for the 4000-ppm females at week 1. Body weights and body weight gains of the high-dose males and females were significantly ($p \leq 0.01$) less than the controls beginning at week 1. For high-dose males and females, absolute body weights were 77-80% and 76-83%, respectively, of the control group levels throughout the study. Body weight gains by high-dose males and females were 29% and 9%, respectively, of the control group levels during the first week of the study and 67% and 57% ($p \leq 0.05$ for both), respectively, of the control group levels overall.

TABLE 2: Selected body weights and body weight gains of male and female rats administered IM-0 in the diet for 13 weeks (g)

Week of study	0 ppm	160 ppm	800 ppm	4000 ppm	20,000 ppm
Males					
0	193.4	196.0	198.5	196.5	192.7
1	267.5	269.6	273.9	270.9	214.4** (80)*
3	379.5	378.9	394.0	387.3	295.4** (78)
6	484.5	484.2	511.2	496.9	380.1** (78)
8	524.7	524.9	557.1	540.7	412.1** (79)
10	563.5	559.3	598.1	580.8	436.3** (77)
13	585.4	578.3	622.7	613.0	455.3** (78)
Wt. gain week 1	74.1	73.6	75.4	74.4	21.8** (29)
Wt. gain 1-6	291.1	288.1	312.7	300.4	187.4** (64)
Wt. gain 1-13	392.0	382.3	424.2	416.5	262.6** (67)
Females					
0	149.0	150.0	151.0	150.0	147.0
1	179.2	176.5	177.3	173.4	149.6** (83)
3	223.9	216.9	224.2	217.5	178.5** (80)
6	272.5	256.0	271.7	256.1	213.4** (78)
8	291.6	273.4	289.9	275.6	224.5** (77)
10	309.0	289.8	307.7	292.0	238.0** (77)
13	317.6	300.6	318.0	301.3	243.3** (77)
Wt. gain week 1	30.2	26.5	26.2	23.4* (77)	2.7** (9)
Wt. gain 1-6	123.5	106.0	120.7	106.2	66.4** (54)
Wt. gain 1-13	168.6	150.5	167.0	151.4	96.3** (57)

Data taken from Tables 3-6, pp. 40-47, MRID 44988427.

*Number in parentheses is percent of control; calculated by reviewer.

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

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption and food efficiency

Selected food consumption and food efficiency data are given in Table 3. Weekly food consumption values and food efficiencies for the 160-, 800-, and 4000-ppm males and females were similar to the controls throughout the study. Food consumption by the high-dose groups was significantly ($p \leq 0.01$) less than the controls during weeks 1-4, 6, 9, and 13 for males and throughout the study for females. Food consumption values during week 1 for the males and females was 59% and 67%, respectively, of the control group levels. Thereafter, food consumption for the high-dose males ranged from 68% at week 2 to 87% at week 5 of the control values and food consumption for the high-dose females varied from 55% at week 12 to 76% at week 5 of the control values.

Food efficiencies by the high-dose males and females for the first week of the study were 50% and 9%, respectively, of their control group values ($p \leq 0.01$). Thereafter, food efficiencies by the high-dose groups were similar to the controls with the exception of males at week 10 ($p \leq 0.05$; 63% of control).

TABLE 3: Selected food consumption (g/animal/day) and food efficiency (%) data of male and female rats administered IM-0 in the diet for 13 weeks

Week of study	0 ppm	160 ppm	800 ppm	4000 ppm	20,000 ppm
Males					
Fd. cons. wk 1	26.0	26.2	27.4	28.5	15.4** (59)[a]
Fd. cons. wk 3	28.5	28.5	30.3	30.0	23.0** (81)
Fd. cons. wk 6	28.0	28.3	29.3	28.6	24.0** (86)
Fd. cons. wk 10	27.6	27.7	29.4	28.5	24.1
Fd. cons. wk 13	26.1	27.2	28.0	28.2	21.9** (84)
Fd. cons. wk 1-13[b]	27.5	28.0	29.0	29.0	22.7
Fd. eff. wk 1	40.7	40.1	39.3	37.7	20.3** (50)
Females					
Fd. cons. wk 1	18.1	17.2	16.4	16.5	12.1** (67)
Fd. cons. wk 3	18.2	18.0	18.6	17.8	12.8** (70)
Fd. cons. wk 6	18.8	16.7	19.4	17.7	13.2** (70)
Fd. cons. wk 10	17.8	17.5	18.1	17.1	12.1** (68)
Fd. cons. wk 13	17.7	15.5	17.6	17.1	12.6** (71)
Fd. cons. wk 1-13[b]	18.2	17.4	18.3	17.2	12.1
Fd. eff. wk 1	23.8	22.2	22.6	20.2	2.2** (9)

Data taken from Tables 7-8, pp. 48-51, Tables 11-12, pp. 56-59, and Text Table II, p. 31, MRID 44988427.
[a]Number in parentheses is percent of control; calculated by reviewer.
[b]Not subjected to statistical analysis.
Significantly different from control: *$p \leq 0.05$; **$p \leq 0.01$.

2. Compound intake

Overall time-weighted average doses for the 160-, 800-, 4000-, and 20,000-ppm groups were 9.9, 48.9, 250.1, and 1246.6 mg/kg/day, respectively, for males and 11.1, 55.9, 275.9, and 1173.7 mg/kg/day, respectively, for females (see Table 1).

D. OPHTHALMOLOGY

No treatment-related ophthalmologic lesions were observed in any animal. Prior to study initiation, red areas in the cornea were observed in one control male and in one high-dose male.

E. CLINICAL CHEMISTRY

No treatment-related differences in any hematological or clinical chemistry parameter were noted between the treated and control groups of either sex. Statistically significant ($p \leq 0.05$) differences from the control values included reduced platelet count for the 160-ppm males, increased alanine aminotransferase activity for the 4000-ppm males, and increased potassium concentration and alkaline phosphatase activity in 20,000-ppm females.

F. URINALYSIS

No significant differences were observed in urinalysis parameters between the treated and control rats of either sex. High-dose males and females had slightly lower water intake which resulted in slightly decreased urine volume as compared with the controls.

G. SACRIFICE AND PATHOLOGY

1. Gross pathology

No treatment-related lesions were noted at necropsy. Findings occurring at low incidence in both treated and control animals included alopecia, malocclusion, and a red zone on the thymus.

2. Organ weights

Terminal body weights of the 20,000-ppm males and females were significantly ($p \leq 0.01$) less than the controls. For the high-dose males, absolute liver and lung weights were significantly ($p \leq 0.05$) decreased by 14-20% and relative (to body weight) brain, testis, and kidney weights were significantly ($p \leq 0.05$ or 0.01) increased by 19-25% as compared with the control. For the high-dose females relative brain, lung, liver, and kidney weights were significantly ($p \leq 0.05$ or 0.01) increased by 17-29% compared with the controls.

3. Microscopic pathology

Treatment-related microscopic lesions were limited to the kidney in the 4000-ppm males and the 20,000-ppm males and females. The kidney lesion consisted of eosinophilic intranuclear inclusions in the proximal tubular epithelium in both males and females. The incidence rates for these treated groups were significantly ($p \leq 0.01$) increased as compared with the controls. Severity of the lesion was rated on a scale of 1-3 designated slight, moderate, or marked, respectively. The incidence (severity) of the inclusions for the control, 4000-, and 20,000-ppm males was 0/10 (0), 7/10 (1.0), and 10/10 (2.7), respectively, and for the control and 20,000-ppm females was 0/10 (0) and 9/10 (1.8), respectively. This lesion was not observed in the other treated groups.

The study authors noted that the kidney inclusions stained with hematoxylin-eosin stain, but not with Feulgen, methyl green-pyronin, or periodic acid-Shiff and Ziehl-Neelsen stain. Therefore, the inclusion is most likely protein not chromatin or DNA, nuclear protein in a plasma cell, carbohydrate, or fat, respectively. Electron microscopic examination showed the ultrastructure of the nucleus to be normal, except for the inclusions.

III. DISCUSSION

A. DISCUSSION

Treatment with the test article did not result in mortalities or cause clinical signs of toxicity in male or female rats. No effects on body weight, body weight gains, or food consumption were observed in either sex at dietary concentrations ≤4000 ppm. For the 20,000-ppm males and females decreased food consumption corresponded with decreased body weight gain especially towards the beginning of the study. Reduced body weight gains by the 20,000-ppm males and females were pronounced during the first week of the study and resulted in lower absolute body weights for these animals throughout the remainder of the study. The reductions in food consumption were possibly due to a lack of palatability. However, reduced food efficiency for the 20,000-ppm males and females during the first week of the study suggests a systemic effect on body weight gain in addition to reduced food consumption.

At terminal sacrifice, differences in absolute and/or relative organ weights were considered a result of differences in final body weights of the 20,000-ppm groups as compared with the controls. Differences in hematological or clinical chemistry parameters between the treated and control groups were sporadic and not dose-related.

A microscopic lesion in the kidney was the main effect of test article administration. Both the incidence and severity were increased for males in the two highest dose groups and for females at the highest dose. Differential staining indicated that the inclusions were protein, but the cause is unknown; the ultrastructure of the nucleus was otherwise normal. Clinical chemistry and urinalysis parameters did not indicate physiological

damage to the proximal tubules. Therefore, the biological significance of this lesion is unknown.

Therefore, the LOAEL for male rats is 4000 ppm (250.1 mg/kg/day) based on an increased incidence and severity of eosinophilic intranuclear inclusions in the proximal tubular epithelium of the kidney. The LOAEL for female rats is 20,000 ppm (1173.7 mg/kg/day) based on decreased body weights, body weight gains, food consumption, and food efficiency and an increased incidence of eosinophilic inclusions in the kidney. The NOAELs for males and females are 800 ppm (48.9 mg/kg/day) and 4000 ppm (275.9 mg/kg/day), respectively.

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

B. STUDY DEFICIENCIES

No deficiencies were noted in the conduct of this study.

DER #16

NI-25 (Acetamiprid): Dermal Absorption
Covance Laboratories. 1997. MRID No. 44651858

Data Evaluation Report

Chemical NI-25 (Acetamiprid)

Study type Dermal absorption Guideline 85-3

Citation

Dermal absorption of ^{14}C NI-25 in male rats (Preliminary and Definitive Phases)
T. Cheng. Covance Laboratories. Covance 6224-234, Protocol No. MC-5577. Oct 3 1997. MRID 446518-58

Reviewed by Robert P. Zendzian PhD 5/30/02
Senior Pharmacologist

Core Classification **Acceptable Guideline**

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 (63.9-75.8, 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/cm^2			119 ug/rat 9.53 ug/cm^2			1,130 ug/rat 90.2 ug/cm^2		
(hours)	%	ug/rat	ug/cm^2	%	ug/rat	ug/cm^2	%	ug/rat	ug/cm^2
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

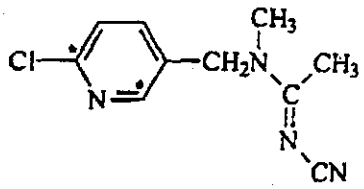
Absorption was small and increased with duration of exposure. The quantity absorbed increased with dose but the percent absorbed increased between the low and intermediate doses and decreased between the intermediate and high doses. This is an unusual pattern.

233

Sections **Materials** through **Sample Collections** are abstracted from the report

Materials

"Radiolabeled Test Material ^{14}C NI-15 (Acetamiprid)

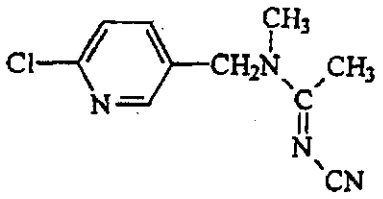


* signifies the position of the radiolabel

Chemical name	N^1-[(6-chloro-3-pyridyl) methyl]-N^2-cyano-N^1-methylacetamide
Molecular formular	$C_{10}H_{11}ClN_4$
Specific Activity	50mCi/mmol
Physical state	Liquid in ethyl acetate

Nonradiolabeled Test Material EXP-80667A [(NI-25 Wettable Powder 70% (w/w)

Chemical name	N^1-[(6-chloro-3-pyridyl) methyl]-N^2-cyano-N^1-methylacetamide
Molecular formular	$C_{10}H_{11}ClN_4$
Physical State	Beige powder



The radiolabeled and nonradiolabeled test materials were provided by the Sponsor."

Stability

"^{14}C NI-25 was stable while refrigerated overnight in the dose solution, and therefore, was stable for the duration of the dosing period. "

Storage

"EXP-80667A (NI-25 Wettable Powder) was stored at room temperature. Technical grade NI-25 (Lot No. NFG-02) was stored at approximately 2° to 8° C. Radiolabeled NI-25 was stored at approximately -20°C. Dosing solutions for Groups I and 2 were prepared on the day of dosing and were stored at room temperature. Dosing solutions for Groups 4, 5, and 6 were prepared the

day before dosing and were stored at approximately 2° to 8° C.."

Test Animal

"Male Crl:CD(SD)BR rats were obtained from the Raleigh, North Carolina facility (preliminary phase) and from the Hollister, California (definitive phase) of Charles River Laboratories, Inc., of Wilmington, Massachusetts. Animals were received on March 25, 1997, for the preliminary phase, and on April 10, 1997, for the definitive phase. The animals were approximately 8 weeks old upon arrival and weighed 176 to 216 g (preliminary phase), and 143 to 203 g (definitive phase). "

Experimental Design

"The 82 male rats on test were assigned to control or treatment groups as follows.

Phase	Group	Number of Animals	Target Dose (mg/animal)	Volume Applied/Animal (uL)
Preliminary	1	4	0.0125	100
Preliminary	2	4	1.25	100
Definitive	3	2	0[a]	100
Definitive	4	24	0.0125	100
Definitive	5	24	0.125	100
Definitive	6	24	1.25	100

a Control group received only the vehicle."

Dose Selection

"Acetamiprid use rates in the field may range from 0.05 to 0.25 lb active ingredient/acre with varying spray volumes ranging from 2 to 1500 gal/acre. Tank mix concentrations range from 0.00005 to 0.05 lb active ingredient/gal. The highest concentration of acetamiprid in a tank mix corresponds to the use rate of 0.1 lb active ingredient/acre in 2 gal/acre (cotton use). It provides a concentration of 0.05 lb/gal or 5.98 mg/mL in the tank mix. Based on this concentration, the high dose of this dermal penetration study was targeted at 1.25 mg/rat (0.1 mg/cm', based on dose volume of 0.1 mL and dose application area of 12.5 cm^2 . The highest tank mix concentration of 5.98 mg/mL corresponds to 0.048 mg/cm^2). Additional lower doses were at log intervals of 0. 125 mg/rat (0.0 1 mg/cm^2) and 0.0 125 mg/rat (0.001 mg/cm^2)."

Dose Preparation and Verification

"Dose solutions were prepared by combining known amounts of "C NI-25, EXP-80667A wettable powder (70% acetamiprid), and 1.0% carboxymethylcellulose (CMC). The carrier, 1.0% CMC, was used for Group 3 (control). Components were mixed by magnetic stirring and vortex-mixing. Radioactivity levels, homogeneity, and radiochemical purity were determined

after preparation. The dose solutions were stored with constant stirring at room temperature if prepared the day of dosing or were stored at approximately 2° to 8°C, prior to dosing.

Aliquots collected predose and postdose were analyzed to confirm radioactivity levels, homogeneity, and radiochemical purity.

Group	^{14}C NI-25 (mg)	EXP-80667A[a] (mg)	NI-25[b] (mg)	1% CMC (ML)	Dose Concentration (mg NI-25/mL)
1	0.263	0	0	2	0.132
2	0.523	34.768	24.720	2	12.6
3	0	0	0	4	NA
4	0.546	0	0	4	0.137
5	2.851	3.330	2.368	4	1.30
6	2.805	66.478	47.266	4	12.5

NA Not applicable.
CMC Carboxymethylcellulose.
a. EXP-80667A contains 71.1% of the active ingredient (NI-25).
b. Calculated amount of NI-25 from formulation of EXP-80667A."

Dose Administration

"At least 16 hours before dosing, the back and shoulders of each animal were shaved, and the shaved area was washed with water. Care was taken not to abrade the skin. The site for application of the test material was defined and protected by a rectangular plastic enclosure (approximately 12.5 cm ^{2}), which was affixed to the back of each rat with cyanoacrylate-based glue. A 100% silicone sealant was applied on the outside of the enclosure and an Elizabethan collar was placed on each animal's neck to protect the dose application site. On the day of dosing, the collar was removed from each animal and the enclosure was inspected for secure attachment."

"The radiolabeled dosing solutions were mixed using a vortex mixer before doses were administered or aliquots were taken. Approximately 0.1 mL of the dosing solution was applied within the enclosure along the midline of the skin site. The weight of the dosing syringe was recorded before and after dosing. The test material was spread evenly across the surface of the skin site using a glass rod (spreader). The glass rod was then rinsed with approximately 3 mL of ACN:water (70:30 v/v) and wiped with a gauze pad; the rinse and wipe were collected for analysis. Duplicate predose and postdose aliquots were taken for dose verification. After test material application, the top of the enclosure was covered with a nonocclusive filter paper cover, and an Elizabethan collar was placed on each animal's neck to protect the dose site."

Skin Wash (Pre-Sacrifice)

"The skin wash occurred immediately before the scheduled sacrifice. Approximately 10 to 15 minutes prior to the scheduled skin wash, the rats were anesthetized with ketamine via an intramuscular injection to the thigh at 0.8 mL/kg. The Elizabethan collar was removed. The nonocclusive filter paper cover was removed from the plastic enclosure and placed in a 100-mL collection container. Twenty-five gauze pads and four cotton-tipped applicators were removed from a prelabeled, pretared 1,000-mL plastic container. The gauze pads were moistened by alternately immersing in either a 2% Ivory soap solution or water. The dose application site was washed using the gauze pads and the cotton-tipped applicators. Following the skin wash, the gauze pads and cotton-tipped applicators were returned to their original container, covered with 100 mL of ACN:water (70:30 v/v), and saved for radio analysis."

Sample Collections

"The accumulated postdose feces and urine from each animal were collected in plastic containers. Immediately following the skin wash, all animals were anesthetized with halothane. The definitive phase animals were then exsanguinated by cardiac puncture, and 2 to 10 mL of blood was collected into heparinized tubes. Residual urine was collected from the urinary bladder and added to the urine sample. For both phases, the skin from the dose site (enclosure included) was excised and collected, and the residual carcass was retained. After excreta collection, cages were washed with [ACN: 1% trisodium phosphate solution (TSP) (60:40 v/v)] and wiped with gauze pads (cage wipes). All samples collected were retained for radio analysis."

Preliminary Phase (Groups 1 and 2). " Urine and feces were collected from 0 to 0.5 hours postdose. At sacrifice, the nonocclusive cover, enclosure, skin wash, cage wash and wipe, skin at application site, and carcass were collected from each animal."

Definitive Phase (Group 3 - Control). " Urine and feces were collected from control animals at 24 hours postdose. Urine samples were surrounded by wet ice. At sacrifice, the nonocclusive cover, enclosure, skin wash, blood, cage wash and wipe, residual urine from the bladder, skin at application site, and carcass were collected from each animal."

Definitive Phase (Groups 4, 5, and 6). " Urine and feces were collected from four animals per group per time point (0.5, 1, 2, 4, 10, and 24 hours postdose sacrifice times). Urine samples from the 24-hour postdose animals were surrounded by wet ice at collection. At sacrifice (4 rats/time point), the following were collected from each animal: nonocclusive cover, enclosure, skin wash, blood, cage wash and wipe, residual urine from the bladder, skin at application site, and carcass."

Results

No abnormalities were observed in the experimental animals during the course of the study.

Actual doses were as follows:

Group	Mean dose levels (mg/rat)	(ug/cm^2)
1	0.0128	1.03
2	1.26	101
4	0.0136	1.09
5	0.119	9.53
6	1.13	90.2

The dose distribution of groups 4, 5 and 6 (the defintive study) are summarized in Table 1. Values are the means of four rats.

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 (63.9-75.8, 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 (21.7-32.2, 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/cm^2			119 ug/rat 9.53 ug/cm^2			1,130 ug/rat 90.2 ug/cm^2		
(hours)	%	ug/rat	ug/cm^2	%	ug/rat	ug/cm^2	%	ug/rat	ug/cm^2
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.

23 X

Table 1. Dermal absorption of NI-25 (Acetamiprid) in the male rat. Mean dose distribution in four rats.

Exposure (hours)	Cover and Encl Rinse	Skin Wash	Skin Test Site	Blood	Carcass	Cage Wash	Cage Wipe	Urine	Feces	Absorbed	Recovery
1.09 ug/cm²											
0.5	0.32	69.7	28.9	NC	NC	NC	NC	NC	NC	NC	99.0
1	1.27	72.0	23.0	NC	0.32	NC	NC	NC	NC	0.33	96.6
2	0.46	75.8	21.7	NC	0.28	NC	NC	0.03	NA	0.33	98.7
4	0.33	67.9	32.2	0.02	0.86	NC	NC	0.31	NC	1.20	98.5
10	0.53	63.9	32.2	NC	0.89	0.10	NC	0.48	NC	1.48	98.1
24	0.36	66.6	25.9	0.03	1.18	0.35	0.16	2.17	0.39	4.27	97.1
9.53 ug/cm²											
0.5	0.32	76.3	22.4	NC	0.16	NC	NC	NC	NC	0.16	99.2
1	0.33	77.7	23.8	0.02	0.56	NC	NC	0.04	NC	0.63	102
2	0.25	77.9	20.9	0.01	0.38	NC	NC	0.03	NC	0.45	99.5
4	0.53	78.8	20.8	0.03	0.77	NC	NC	0.19	NC	1.02	101
10	0.30	69.2	26.5	0.06	2.32	0.14	0.11	1.43	NC	4.07	100
24	0.89	64.9	25.0	0.05	1.79	0.37	0.24	3.25	0.65	6.34	97.1
90.2 ug/cm²											
0.5	0.31	80.4	16.9	0.01	0.32	NC	NC	NC	NC	0.34	98.0
1	1.90	84.0	11.9	NC	0.14	NC	NC	0.01	NC	0.16	98.0
2	0.19	87.5	10.2	0.01	0.23	NC	NC	0.02	NC	0.27	98.1
4	0.45	80.3	15.4	0.01	0.44	0.02	NC	0.16	NC	0.64	96.8
10	0.57	80.9	15.2	0.01	0.51	0.04	0.04	0.17	NC	0.78	97.4
24	0.34	79.3	14.5	0.02	0.77	0.20	0.12	1.41	0.30	2.82	97.0

NC not calculated. mean is not calculated when a group has 2 or more nondetected values and/or <0.005 values
Absorbed is the sum of blood, carcass, cage wash, cage wipe, urine and feces

HED Records Center Series 361 Science Reviews - File RM#313 - Page 230 of 283

Health Canada | Santé Canada

Pest Management Regulatory Agency | Agence de réglementation de la lutte antiparasitaire

2250 promenade Riverside Drive
Ottawa, Ontario
K1A 0K9

Telephone/Téléphone:
Fax/Télécopieur:

Your file *Votre référence*

Our file *Notre référence*

July 6, 2001

Memorandum To/Note adressée à: Hemendra Mulye, Science Team Lead
EAD

From/De: Dana Bruce
OEAS, HED

Subject/Objet: **Sub. No(s):**
1999-2081,1999-2087, 1999-2088, 1999-2089, 1999-2090
Product Name:
Chipco Brand Tristar 70 WSP Insecticide
Assail Brand 70 WP Insecticide
Adjust Brand 70 WP Insecticide
Pristine Brand RTU Insecticide
Active Ingredient: Acetamiprid
Applicant: Rhone Poulenc

ACTION REQUESTED: **Secondary Review of U.S. EPA Data Evaluation Report for *in vivo* dermal absorption study (DACO 5.8)**

Study Citation:

Dermal absorption of ^{14}C NI-25 in male rats (Preliminary and Definitive Phases). T. Cheng. Covance Laboratories. Covance 6224-234. Protocol No. MC-5577. Oct 3 1997. MRID 446518-58.

Secondary Review Comments:

During the secondary review of the above-noted study, the following observations were made:

1. No comparison was made between the composition of the proposed formulations and the study test material. This is required to assess the relevance of the study to the proposed formulations. To protect confidential business information, this information is typically a separate appendix to the U.S. EPA DERs. However, given the joint review status of these submissions, sharing of this information would be appropriate.

2. Percent dermal absorption is reported as the sum of blood, carcass, cage wash, cage wipe,

urine and faeces. Residues retained at the skin site are reported separately. As the study design did not permit analysis of the fate of skin bound residues, PMRA proposes that the percent absorbable should be deemed to include residues retained at the skin site. This would be consistent with guidance provided in U.S. EPA Health Effects Test Guidelines OPPTS 870.7600 (Dermal Penetration).

3. In the Summary and the Results sections, the value 63.9 should replace 63.6 for the minimum percent of dose in the skin wash for the low dose. For the skin residue results, the value 32.2 should replace 29.1 for the low dose.

_____Date:_____
Dana Bruce
Evaluation Officer, OEAS

_____**Date:**_____
Christine Norman
Section Head, OEAS

DER #17

NI-25 (Acetamiprid): Metabolism and Pharmacokinetics - DRAFT
Nippon Soda. 1995-1997. MRID Nos. 44988503 thru -07

DATA EVALUATION RECORD

ACETAMIPRID

STUDY TYPE: METABOLISM AND PHARMACOKINETICS – RAT
[OPPTS: 870-7485 (§85-1)]
MRID 44988503, 44988504, 44988505, 44988506, 44988507

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 01-78 H-L

Primary Reviewer:
Robert A. Young, Ph.D., D.A.B.T. Signature: _____
Date: _____

Secondary Reviewers:
H. Tim Borges, Ph.D., MT(ASCP), D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed by UTBattelle, LLC, for the U.S. Dept. of Energy under contract DEAC0500OR22725

ACETAMIPRID DRAFT **Metabolism Study [OPPTS 870.7485 (§85-1)]**

EPA Reviewer: Pamela M. Hurley, Ph.D. _____, Date _____
Registration Action Branch 2 (3002)
EPA Work Assignment Manager: SanYvette Williams-Foy, D.V.M. _____, Date _____
Registration Action Branch 2 (3002)

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - Rat [OPPTS 870.7485 (§85-1)]

DP BARCODE: D264156
P.C. CODE: 099050

SUBMISSION CODE: S575947
TOX. CHEM. NO.: NA

TEST MATERIAL (PURITY): NI-25 (>99.9%)

SYNONYMS: Acetamiprid; NI-25; (*E*)-N^1-[(6-chloro-3-pyridyl)methyl-N^3-cyano-N^1-methylacetamidine; MOSPILAN®

CITATION: 1) Tanoue, T., Mori, H. (1997). ^{14}C-NI-25 - Metabolism Study in Rats (A Summary Report). Nisso Chemical Analysis Co., Ltd. (NCAS), Odawara Laboratory, 345 Takada, Odawara, Kanagawa 250-02, Japan. NACS Report No. EC-912. September 25, 1997. MRID 44988503. Unpublished.

2) Tanoue, T., Mori, H. (1997). ^{14}C-NI-25 - Metabolism Study in Rats. Nisso Chemical Analysis Co., Ltd. (NCAS), Odawara Laboratory, 345 Takada, Odawara, Kanagawa 250-02, Japan. NCAS Report No. EC-724. March 31, 1997. MRID 44988505. Unpublished.

3) Premkumar, N., Guo, C., Vengurlekar, S. 1995. Absorption, Distribution, Metabolism, Elimination, and Pharmacokinetics After Chronic Dosing of [^{14}C]-NI-25 in Rat. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri, U.S.A. 65202. Study No. 42207. March 24, 1995. MRID 44988506. Unpublished.

4) Tanoue, T., Mori, H. (1997). ^{14}C-NI-25 - Metabolism Study in Rats (Qualitative and Quantitative Analysis of Metabolites in Group C). Nisso Chemical Analysis Co., Ltd. (NCAS), Odawara Laboratory, 345 Takada, Odawara, Kanagawa 250-02, Japan. NCAS Report No. EC-842-1. March 27, 1997. MRID 44988504. Unpublished.

5) Premkumar, N., Guo, C. 1995. [^{14}C]-NI-25 - Biliary Excretion in Rat. ABC Laboratories, Inc., 7200 E. ABC Lane, Columbia, Missouri, U.S.A. 65202. Study No. 42206. March 17, 1995. MRID 44988507. Unpublished.

SPONSOR: Nippon Soda Co., Ltd., Product Development Dept. Agro Products Division, 2-2-1 Ohtemachi, Chiyodaku, Tokyo 100, Japan,

EXECUTIVE SUMMARY: Studies were conducted to assess the metabolism and disposition of orally and intravenously administered NI-25 (acetamiprid) in male and female Sprague-Dawley rats. Experiments included single oral or i.v. dose (MRID 44988505) using groups of 5 to 10 rats and doses of 1 or 50 mg/kg of pyridine ring-labeled [^{14}C]-NI-25 (Lot no. EC-09-09, EC-09-10, radiochemical purity 98.9%, chemical purity >99%), and a single 1 mg/kg oral dose group (MRID 44988505) using cyano-labeled [^{14}C]-NI-25 (Lot no. EC-09-21C, radiochemical purity 98.6-99.2%, chemical purity >99%). An additional study (MRID 44988506) utilized a 15-day repeat-dose protocol in which groups of 3-5 male and female Sprague-Dawley rats were given 1 mg/kg/day doses of ring [^{14}C]- NI-25 (Lot no. CFQ8019, radiochemical purity 97%, chemical purity >99%) or non-labeled NI-25 (Lot no. NNI-01, purity >99.9%) and terminated at 1, 10, or 96 hours after the last dose. Vehicle controls received equivalent volumes of 0.9% saline. A biliary excretion study (MRID 44988507) using 4 male and 4 female Sprague-Dawley rats (with saline controls) was conducted using ring-labeled [^{14}C]-NI-25 (Lot no. CFQ8019, radiochemical purity 97.1%, chemical purity >99%) and non-labeled NI-25 (Lot no. NNI-01, purity >99.9%). A summary report (MRID 44988503) provided an overview of the findings of the other reports that assessed the absorption, distribution, metabolism and excretion of NI-25 (acetamiprid). A study to characterize urinary and fecal metabolites (MRID 44988504) utilized biological samples generated by a 1 mg/kg single-dose group reported in MRID 44988506.

There were no treatment-related toxicologic effects. Recovery of administered radioactivity for the various experimental groups in the repeat-dose study (MRID 44988506) was 91.7-106% (except Group V which was 71.7-85.% due possibly to loss of urine sample which contained substantial radioactivity), 93-100% for the single-dose study (MRID 44988505),and 89.6-94.5 for the biliary excretion study (MRID 44988507). Overall, these mass balance data are considered acceptable.

Absorption of orally administered NI-25 was rapid and complete based upon urinary excretion data and intravenous administration data. Estimation of absorption by comparison of urinary excretion following intravenous and oral administration (i.e., [urinary excretion oral/urinary excretion, i.v.] x 100) indicated 96-99% absorption following oral administration. This was consistent with urinary excretion, cage wash, and tissue/body burden data from MRID 44988506, showing ~65 - 75% absorption. There did not appear to be any biologically relevant gender-related differences in any of the groups.

Urinary excretion was the major route of elimination of [^{14}C]- NI-25 (acetamiprid). Excretion following a single oral dose of NI-25 was rapid regardless of dose or label position with the majority (76-97%) of the urinary excretion occurring within 24 hours. Urinary excretion following intravenous administration was similar to that for the oral route. Repeat dosing (MRID 44988506) also resulted in rapid and complete urinary excretion (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. Fecal excretion of radioactivity by rats in the biliary elimination study was expectedly less; 6.72% (males) and 5.84% (females) than that for the other experimental groups. Biliary elimination data (MRID 44988507) exhibited considerable variability, although mean biliary excretion of radioactivity did not vary notably between genders. Over the 48-hour period, biliary elimination accounted for approximately 19% of the administered radioactivity.

Pharmacokinetic parameters reflected the rapid absorption and excretion of the NI-25. Peak concentrations occurred within 1-2 hours for the low- dose (1 mg/kg) groups and only slightly later (~4 hrs) for the high-dose (50 mg/kg) group. Clearance from the blood was nearly complete within 48 hours. Tissue half-lives ranged from 3.5 - 5.9 hours for males and 2.9 - 5.9 hours for females in the low-dose group, and 6.0 - 8.5 hours for males and 6.3 - 8.3 hours for females in the high-dose group. These data suggest that elimination from tissues was not greatly affected by a 50-fold dose increment. Consistent with rapid and complete excretion, the time-course in tissues was similar to that for blood. There was no evidence for sequestration of administered radioactivity and no toxicologically significant gender-related differences. Pharmacokinetic parameters derived from the 15-day repeat dose study (MRID 44988506) were similar to those from the single-dose metabolism study.

Tissue distribution data for the 15-day repeat-dose study (MRID 44988506) showed a wide volume of distribution but tissue burdens were low (generally <1% of the administered dose). The greatest radioactivity was expectedly found in the gastrointestinal tract (including lumen contents), where up to 3-4% of the administered dose was detected in Group I (single oral 1 mg/kg dose). Liver and kidney also exhibited somewhat greater levels of radioactivity than did other tissues but did not exceed 0.66% of the dose and declined notably from 1 hour to 96 hours following the last of 15 doses. At 96 hours after the final dose (Groups II and IV, MRID 44988506), radioactivity levels in all tissues generally represented considerably less than 0.001% of the administered dose. There was no significant difference between whole blood radioactivity and plasma radioactivity. No gender-related differences were observed. The data indicate that 15-day repeat doses of 1 mg/kg do not result in tissue sequestration of the test article or its metabolites.

Under the conditions of the reported experiments, NI-25 (acetamiprid) 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 (Group IV of MRID 44988506) 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. 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, 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 of the glycine conjugate. A metabolism pathway was proposed by the study authors of the ADME study (MRID 44988505) that is consistent with available data from the reviewed studies.

These metabolism/kinetics studies (MRID 44988503, 44988504, 44988505, 44988506, and 44988507) in rats are collectively **Acceptable/Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)].

248

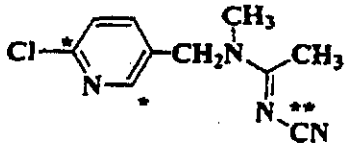
COMPLIANCE: Good Laboratory Practice Compliance Statements, and signed and dated Quality Assurance statements were provided in the study reports.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test compound

Radiolabeled:[^{14}C]-acetamiprid ([pyridine-2,6-^{14}C]NI-25; abbreviated as ring-^{14}C-NI-25 or ^{14}C-NI-25)
[cyano-^{14}C]NI-25 (abbreviated as CN-^{14}C-NI-25)
Batch/Lot No.: CFQ8019 (Ref. no. R-156) (MRID 44988506; 44988504)
EC-09-09 (low dose), EC-09-10 (high dose) (MRID 44988505)
Specific Activity: 33 mCi/mmol (MRID 449885-6)
23.2 mCi/mmol (low dose, MRID 44988505;44988506)
1.02 mCi/mmol (high dose, MRID 44988505)
33 mCi/mmol (MRID 44988507)
Radiochemical purity: 97.1-97.% (MRID 44988506; 44988504; 44988507)
98.9% (low dose, MRID 44988505)
99.3% (high dose, MRID 44988505)
Chemical purity: >99.9% (MRID 44988506;44988504; 44988505; 44988507)
Description: Supplied as solution in methanol:methylene chloride
Contaminants: None noted
Structure:



(*labeled position for ring -^{14}C-NI-25)
(**labeled position for CN-^{14}C-NI-25)

Non-radiolabeled: acetamiprid
Lot No.: NNI-01
Purity: >99.9%
Description: solid
Contaminants: none noted
CAS No.: 135410-20-7

2. Vehicle

Saline (0.9%) was used as the dose vehicle. For the high-dose groups in MRID 44988505, the test article was suspended in 1% carboxymethylcellulose.

3. Test animals

Species: rat
Strain: 1) Sprague-Dawley (Hilltop Lab Animals, Inc., Scottdale, PA) for MRID 44988506; 44988504; 44988507)
2) SD (Crj:CD) Charles River Japan, Atsugi Farm, Kanagawa, Japan) for MRID 44988505.
Age and weight at study initiation:
Age: 5.5 - 6 weeks at dosing (includes 7-day acclimation period);~10-12 weeks (MRID 44988507)
Weight: 222.3 - 294.2 g (males), 162.0 - 202.7 g (females) at dosing (MRID 44988503); 193 g (males) and 149 g (females) in blood kinetics experiments; 181 g (males) and 145 g (females) for excretion kinetics experiments (MRID 44988504; 44988506)
(276.3 - 298.7 g males; 210.0 - 224.5 g females)at experimentation
Housing: 1) Housed in groups (number not specified) in polycarbonate cages during acclimation; housed individually in stainless steel metabolism cages during metabolism studies (MRID 44988503)
2) Housed individually in metabolism cages (KN-646B, Natsume) (MRID 44988504; 449885-6)
Diet: Certified Rodent Diet (Checkers PMI 5002, Bourn Feed and Supply Co., Columbia, MO) *ad libitum* (MRID 44988503; MRID 44988507)
Diet MF, Oriental Yeast, Lot nos. 93.02.04 B1 and 93.07.05.B3 *ad libitum* (MRID 44988504; 44988506)
Water: Tap water provided *ad libitum* (all studies; autoclaved distilled for MRID 44988507)
Environmental conditions:
Temperature: 20.3±0.5°C (MRID 44988503)
21-26°C (MRID 44988504, 44988506)
23±0.43°C (MRID 44988507)
Humidity: 49.6±6.9% (MRID 44988503)
40-60% (MRID 44988504, 44988506)
42±4.5% (MRID 44988507)
Air changes: 13.7/hr (MRID 44988503)
10/hr (MRID 44988504, 44988506)
15.3/hr (MRID 44988507)
Photoperiod: 12 hrs/12 hrs (all studies)
Acclimation period: 7 days (MRID 44988503)
1.5 weeks (MRID 44988504, 44988506)
Not specified for MRID 44988507

4. Preparation of dosing solution

For MRID 44988506 and 44988504 a dosing solution of non-labeled analytical grade NI-25 (72.5 mg) and 0.9% saline (q.s. to 100 mL) was prepared and aliquots analyzed in duplicate by HPLC for stability on the first day of dosing and the last day of dosing.

For the high doses (50 mg/mL) used in MRID 44988505, the test article was suspended in 1% carboxymethylcellulose due to insolubility in saline at the high concentration required. For the biliary excretion study (MRID 44988507), the dosing solution was formulated as 2.40 g analytical grade NI-25 and 0.9% saline q.s. to 5 mL. A 150 µL aliquot of [^{14}C]-NI-25 was added.

Results –

Homogeneity: For the metabolism/disposition study (MRID 44988505), homogeneity was assessed prior to and immediately following dosing. Coefficient of variation ranged from 1.22 to 6.57%, thereby affirming acceptable homogeneity.

Stability: HPLC analysis conformed stability of the dose solutions during the periods relevant to the studies.

Dose confirmation: Actual administered dose in the repeat-dose study (MRID 44988506; 44988504) was 0.97 - 1.01 mg/kg, an insignificant variance from the target dose of 1.0 mg/kg. For the metabolism study (MRID 44988505), actual low doses ranged from 0.94 - 1.05 mg/kg and high doses ranged from 47.7 - 51. 8 mg/kg; both represented acceptable variability from nominal. For the biliary excretion study (MRID 44988507), administered doses were 1.02 mg/kg (males) and 1.07 mg/kg (females) as compared to the target dose of 1 mg/kg, again indicating acceptable variability.

B. STUDY DESIGN AND METHODS

1. Group arrangements

For the 15-day repeat exposure study (MRID 44988506) on absorption, distribution, metabolism, and excretion (ADME), an in-house developed randomizing program was used to assign rats to the experimental groups (I, II, and III).

TABLE 1. Study design for disposition and metabolism of NI-25 (acetamiprid) in rats

Experiment group	Dose (mg/kg)	Number/Sex	Remarks
Group A	1	5♂; 8♀	Ring-[^{14}C]-NI-25; single intravenous dose; excretion kinetics and quantitative analysis of metabolites (MRID 44988505)
Group B	1	10♂; 10♀	Ring-[^{14}C]-NI-25; single oral dose; blood levels (5 rats each sex), excretion kinetics and quantitative analysis of metabolites (5 rats of each sex)
	1	9♂; 9♀	Ring-[^{14}C]-NI-25; single oral dose; tissue distribution (MRID 44988505)
Group D	50	10♂; 10♀	Ring-[^{14}C]-NI-25; single oral dose; blood levels (5 rats each sex), excretion kinetics and quantitative analysis of metabolites (5 rats each sex)
	50	9♂; 9♀	Ring-[^{14}C]-NI-25; single oral dose; tissue distribution (MRID 44988505)
Group CN-B	1	10♂; 10♀	[CN-^{14}C]NI-25; single oral dose; blood levels (5 rats each sex); excretion kinetics and quantitative analysis of metabolites (5 rats each sex) (MRID 44988505)
Group C	1	5♂; 5♀	Metabolite analysis of samples from repeat dose Group IV of MRID 44988506; reported in MRID 44988504
BII	1	4♂; 4♀	Biliary excretion study (MRID 44988507)
BI	0	1♂; 1♀	Saline (0.9%) controls for biliary excretion study (MRID 44988507)
I	1	3♂; 3♀	[^{14}C]-NI-25 for 15 days; ADME; sacrificed at 1 hr (MRID 44988506)
II	1	3♂; 3♀	[^{14}C]-NI-25 for 15 days; ADME; sacrificed at 10 hrs (MRID 44988506)
III	1	3♂; 3♀	[^{14}C]-NI-25 for 15 days; ADME; sacrificed at 96 hrs (MRID 44988506)
IV	1	5♂; 5♀	Non-labeled NI-25 for 14 days followed by single dose of [^{14}C]-NI-25 on Day 15; ADME; sacrificed at 96 hrs; (MRID 44988506)
V	1	5♂; 5♀	Non-labeled NI-25 for 14 days followed by single dose of [^{14}C]-NI-25 on Day 15; ADME; sacrificed at 48 hrs; (MRID 44988506); resulting sample matrices used for quantitative/qualitative analysis of metabolites (MRID 44988504)
VI	0	2♂; 2♀	0.9% saline controls; sacrificed at 96 hrs; (MRID 44988506)

Information taken from p. 14, MRID 44988505, p. 18, MRID 44988506, p. 16, MRID 44988507

2. Dosing and sample collection

Test animals were dosed orally (1 mg/kg via gavage feeding needle) with dose volumes based upon Day -1 body weights, average body weight increases over the treatment period, and the last treatment day for the final dose. Dose volumes ranged from 0.23 - 0.41 mL/day (males) and 0.14 - 0.29 mL/day (females). Samples were stored at ~ -20°C and express shipped to sponsor on dry ice.

Expired air – Not collected. Results of preliminary experiments (Appendix A of MRID 44988505) indicated that no radioactivity was detected in expired air for 48 hours after dosing.

Blood – In the repeat dose study (MRID 44988506), whole blood was taken in heparinized capillary tubes from the tail vein. For Group IV (MRID 44988506), samples were taken one hour after administration of saline on Days 1 and 15. Blood was collected one hour post dosing on Days 1, 3, 7, and 15 for Group III, and at 0.25, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 24, and 48 hours dosing from rats in Group V. Blood was collected from the descending vena cava of all rats in Groups I, II, II, IV, and VI at the time of sacrifice. Blood volume was recorded and 50 µL in heparinized capillary tubes analyzed for radioactivity. The same sampling intervals and procedures were used for MRID 44988505 except that blood was collected in heparinized capillary tubes from clipped tail tips.

Bile – Bile was collected continuously from rats in the biliary excretion study (MRID 44988507). Collection vials were replaced at 3, 6, 12, 24, and 48 hours. Volumes of all samples were recorded and samples stored frozen.

Feces – For MRID 44988506, feces were collected from Groups I, II, and III from Day -1 and at 24-hour intervals thereafter. Day -1 samples were kept separate but samples from the following days were pooled within individual rats with the exception of Day 15 samples from Groups I and II, and Day 15-19 samples from Group III which were also kept separately. For Groups IV, V, and VI, feces were collected on Day 14 and at 24-hour intervals after the administration of the test article. In the metabolism study (MRID 44988505), feces were also collected daily. Feces were collected at 24 and 48 hours in the biliary excretion study.

Urine – Urine samples were collected and pooled as described for feces.

Cage wash – Cage wash (500 mL of distilled water followed by 500 mL of acetonitrile) was collected and homogenized. Triplicate aliquots (1 mL) were analyzed by LSC. In the biliary excretion study, 30 mL of distilled water was used to wash the cage of each rat.

Tissues/Carcass – Liver, kidney, lung, pancreas, heart, spleen, brain, testes/ovaries, skeletal muscle, inguinal fat, skin, thyroid, bone, adrenal glands, and gastrointestinal tract and contents were collected from rats in Groups I, II, and III of the repeat dose study (MRID 44988506). To coincide with times of maximum (t_{cmax}), ½ t_{cmax}, and 1/4 t_{cmax} for blood concentrations, tissues from rats in the metabolism study (MRID 44988505) were taken at 1, 5, and 10 hours after treatment with the low dose and at 5, 14, and 24 hours after treatment with the high dose. Each sample was weighed and stored frozen. For the biliary excretion study, liver and the gastrointestinal tract (with contents) were collected at termination of the experimental period. The carcasses from all studies were retained for solubilization.

3. Sample preparation/analysis

Expired air – Expired air was not collected.

253

Blood – Blood samples were solubilized and counted in triplicate by LSC.

Bile – Aliquots (0.25 mL) were analyzed in triplicate by LSC.

Feces – Feces were weighed and stored until analysis. For Groups I, II, and III, Day 1-14 samples (MRID 44988506) were homogenized over liquid nitrogen while remaining fecal samples were ground with a liquid nitrogen-cooled mortar and pestle. Homogenized samples were solubilized, weighed in triplicate followed by radioactivity determination by LSC. For MRID 44988505), samples were lyophilized under vacuum, weighed, powdered and triplicate samples (50-100 mg) combusted and radioassayed.

Urine – Urine samples (plus cage wash) from the repeat dose study (MRID 44988506) were homogenized by shaking and adjusted to 1000 mL with distilled water. Scintillation fluid was added to triplicate 0.5 mL aliquots and radioactivity determined by LSC. Urine and cage wash samples from the metabolism study (MRID 44988505) were filtered through filter paper and the filtrate adjusted to 100 mL. Radioactivity was measured in triplicate samples and the filter paper was combusted and radioassayed.

Cage wash – Triplicate 1-mL aliquots were analyzed for radioactivity. Remaining samples were stored at ≈-20°C..

Tissues/Carcass – For the repeat dose study (MRID 44988506), adrenal glands, thyroids and ovaries were solubilized in Soluene and analyzed by LSC. Frozen liver and gastrointestinal tract/contents were ground to powder in liquid nitrogen using a Polytron homogenizer; other tissues were ground in a mortar and pestle. The powders were later solubilized and counted by LSC. Bone samples were combusted in a Packard 307 Tricarb Sample Oxidizer (Packard Instrument Co., Downers Grove, IL). Oxidizer efficiency was determined and recoveries monitored between every 10 samples. The residual carcasses were weighed and placed in a half- gallon glass jar, solubilized at 65°C in 1 L of a digestion solution (80 g NaOH in water/methanol/ Triton X-405 [600:300:100, v/v/v]) for approximately 24 hours. The carcass solution was then weighed and homogenized, and 200g samples stored at -20°C. Scintillation fluid was added to triplicate aliquots and analyzed by LSC 24 hours later.

In the metabolism study (MRID 44988505), spleen, heart, lung, thyroid, sciatic nerve, ovaries, femoral bone, adrenal glands, and pancreas were directly combusted and lyophilized. Other tissues (fat, liver, kidney, whole blood, testes, brain, muscle, skin, and carcass) were homogenized in water, lyophilized and combusted prior to radio-analysis.

4. Analytical techniques

Liquid Scintillation Counting (LSC) - Radioactivity was measured using a TM Analytic Delta 300® (TM Analytic, Inc. Elk Grove Village, IL) LSC system (MRID

44988506), and Aloka LSC-672 or LSC-903 (MRID 44988505). Various scintillants (Permafluor®, Ultima Gold®, Carbosorb®, Hionic Fluor®, Emulsifier-Scintillator 299®) were used depending on the matrix being analyzed. Counting efficiency was determined using the external standard ratio technique. Samples were counted for 10 minutes or 20,000 counts.

High Performance Liquid Chromatography (HPLC) - HPLC in the repeat dose study (MRID 44988506) was performed using a Varian system and Lichospher 100, RP-18 column (5 µm x 24.4 cm x 4 mm; EMerck, Darmstad, Germany), Varian UV detector and Raytest Ramona-90 ^{14}C detector. Solvent A was 10 mM KH_2PO_4-H_3PO_4 (pH ≈4) in distilled water. Solvent B was acetonitrile. A gradient flow (85%A:15%B to 30%A:70%B at 18-20 minutes and back to 85%A:15%B at 25 minutes) was used with a flow rate of 1 mL/min. In the metabolism study (MRID 44988505), both preparative and analytical HPLC were used, the equipment and procedures for which were adequately described in the study report.

Thin-layer Chromatography (TLC) - For metabolite analysis in MRID 44988505, TLC plates (silica gel 60 F_{254} 20x20 mm, Art. 5554, Merck) were used with three solvent systems: 1) methylene chloride:acetone [6:4]; 2) methylene chloride: methanol:acetic acid [8.5:0.5:1.0]; 3) methylene chloride:methanol: 25% ammonia hydroxide [8.5:1.0:0.2]. Radiolabeled fractions were detected by LSC and nonlabeled fractions were detected by UV light.

Liquid chromatography-mass spectrometry (LC-MS) in conjunction with reference standards was used for identification of metabolites. Metabolite structure was assessed by mS and nuclear magnetic resonance (NMR).

5. Calculations and Statistics

Group means and standard deviations were presented. Pharmacokinetic parameters for the repeat dose study (MRID 44988506) were calculated using MINSQ® and RSTRIP® software (MicroMath Scientific Software, Salt Lake City, UT) with individual animal pharmacokinetic profiles determined as open-compartment, first-order kinetics with no lag time. For the metabolism study (MRID 44988505), kinetic parameters were calculated using least squares linear regression and typical constants ($0.693/\beta$).

Calculations/conversion for radioanalysis data were provided in the study reports.

II. RESULTS

A. DISTRIBUTION/EXCRETION STUDIES

1. Mass balance

Mass balance data for the single-dose groups of the metabolism study (MRID 44988505) are summarized in Table 2. The overall recovery of 93-100% represented an acceptable mass balance accounting of the administered radioactivity.

Overall recovery of administered radioactivity was an acceptable 91.7 - 106% for Groups I, II, III, and IV of the repeat-dose study (MRID 44988506) but was deficient (71.7 - 85.6%) for Group V (Table 3). Total recovery of administered radioactivity was 89.6 - 94.9% in the biliary elimination study (MRID 44988507) (data not shown in this Data Evaluation record).

TABLE 2. Overall recovery of administered radioactivity (% of dose) following a single oral dose (1 or 50 mg/kg) or single intravenous dose (1 mg/kg) in rats[a] (MRID 44988505)

Exp. Group	Expired air	Urine	Feces	Tissues	Total
A (I.v.)					
Males	NA	81.59	15.55	0.63	97.78
Females	NA	79.74	17.04	0.48	97.26
B (Oral)					
Males	NA	81.06	11.64	0.42	93.13
Females	NA	79.33	13.79	0.52	93.64
D (Oral)					
Males	NA	86.43	12.96	0.74	100.13
Females	NA	73.84	17.33	0.58	91.74
Cn-b(oral)					
Males	NA	90.27	5.32	0.96	96.55
Females	NA	88.35	5.19	0.84	94.38

[a] recovery over a 4-day period; n=5

NA: not applicable; expired air found not to be a relevant route of excretion.

Data taken from Tables 3, p. 30, MRID 44988505.

256

TABLE 3. Overall recovery of administered radioactivity (% of dose) from rats following 15-day repeat dosing (1 mg/kg/day) (MRID 44988506)

Exp. Group	Expired air	Urine/Cage Wash	Feces	Final Cage Rinse	Tissues/ carcass[d]	Total
Group I[a]						
Males	ND	53.4±5.25	31.0±0.55	7.58±2.98	9.0	100.9
Females	ND	58.1±5.40	21.9±2.44	10.7±1.40	9.0	99.7
Group Ii[b]						
Males	ND	56.6±6.85	29.8±3.09	7.32±1.94	4.78	98.4
Females	ND	59.3±4.10	25.2±6.00	6.98±2.21	3.97	95.5
Group Iii[c]						
Males	ND	61.4±0.62	32.0±4.08	3.92±0.73	0.062	97.4
Females	ND	56.0±2.42	27.5±1.42	7.93±2.66	0.236	91.7
Group Iv[c]						
Males	ND	64.8±6.99	35.3±5.99	5.86±2.80	0.36	106.3
Females	ND	62.1±5.32	28.7±4.30	11.3±3.72	0.45	102.6
Group V						
Males	ND	38.0±10.6	20.7±9.13	12.1±4.59	0.88[e]	71.6
Females	ND	41.5±14.0	24.3±4.82	18.7±3.62	1.07[e]	85.6

NA: not applicable; expired air found not to be a relevant route of excretion
[a]1 hr, [b]10 hrs; [c]96 hrs
[d] Values are summation of means for individual tissues; S.D. not presented.
[e] Values are for blood and residual carcass only.
Data taken from Tables IX (p. 47) and XII - XVII (pp. 54 - 58), MRID 44988506.

2. Absorption

Absorption of orally administered NI-25 was relatively rapid and complete based upon urinary excretion data and intravenous administration data. Estimation of absorption by comparison of urinary excretion following intravenous and oral administration (i.e., [urinary excretion oral/urinary excretion, i.v.] × 100) showed that absorption following oral administration was 96-99%.

Absorption of the test material, implied from urinary excretion, cage wash, and tissue/body burden data from MRID 44988506, indicated that up to ~65 - 75% of the administered repeated oral dose was absorbed (Table 3). There did not appear to be any biologically relevant gender-related differences in any of the groups and total absorption did not vary significantly at the 1, 10, or 96-hour post-treatment sacrifice times. The available data indicated that absorption was relatively rapid.

3. Excretion

Urinary excretion was the major route of elimination of administered radioactivity in the metabolism study, MRID 44988505 (Table 2). Excretion of radioactivity following a single oral dose of NI-25 was rapid regardless of dose or label position. The majority of the urinary excretion (76-97%) occurred within 24 hours (data not reproduced in this Data Evaluation Record) and nearly complete within 48 hours. As expected, urinary excretion was somewhat greater for the intravenous dose group than for the oral dose groups but the difference was not biologically relevant or indicative

of compromised/saturated absorption processes following oral administration. In the biliary elimination study (MRID 44988507), 24.3% (males) and 36.9% (females) of the administered dose was excreted in the urine of the cannulated rats over a period of 48 hours.

Consistent with the findings of the metabolism study (MRID 44988505), the available data from the repeat dose study (MRID 44988506) also revealed that urinary excretion was the most prevalent route of excretion (Table 3). As shown by excretion data from experimental Groups I, II, III, and IV, total urinary excretion did not vary significantly from 1 to 96 hours post dosing (15-day repeated dose) indicating that most excretion occurred within 24 hours.

Biliary excretion data from the biliary excretion study (MRID 44988507) are shown in Table 4. Considerable variability in biliary output was observed among the bile cannulated rats, although mean biliary excretion of radioactivity did not vary notably between genders. Over the 48-hour period, biliary elimination accounted for approximately 19% of the administered radioactivity.

TABLE 4. Time-course for biliary elimination of radioactivity (% of dose)[a] in rats following a single 1 mg/kg intragastric dose of [^{14}C]-NI-25 (acetamiprid).

Time (hrs)	Males	Females
3	2.1±1.6	2.9±1.6
6	3.1±1.3	4.5±1.9
12	4.7±1.7	6.3±0.9
24	7.1±3.2	3.8±1.8
48	2.9±1.7	1.0±1.3
Total	19.0±1.47	18.6±0.62

[a] Mean ± S.D. of three rats (calculated by reviewer, except total)
Data taken from Table IV, p. 33, MRID 44988507.

Fecal elimination 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 (Table 2). Fecal excretion of radioactivity by rats in the biliary elimination study was expectedly less; 6.72% (males) and 5.84% (females) (data not reproduced in this Data Evaluation Record) than that for the other experimental groups.

4. Tissue distribution

Tissue distribution data for the 15-day repeat-dose ADME study (MRID 44988506) revealed that, although radioactivity was widely distributed, tissue burdens represented relatively small amounts (generally <1%) of the administered radioactivity. The greatest radioactivity was detected in the gastrointestinal tract where up to 3-4% of the administered dose was detected in Group I (Table 5). The greater radioactivity in this tissue could be readily attributed to unabsorbed test material in the lumenal contents which was included in the analysis. Liver and kidney also exhibited somewhat greater levels of radioactivity than did other tissues but did not exceed 0.66% of

the dose and declined notably from 1 hour to 96 hours following the last of 15 doses. At 96 hours after the final dose (Groups II and IV, MRID 44988506), radioactivity levels in all tissues generally represented considerably less than 0.001% of the administered dose. There were no significant differences between whole blood radioactivity and plasma radioactivity. No gender-related differences were observed and there was no evidence for sequestration of the test article or its metabolites.

TABLE 5. Radioactivity (% of administered dose) in selected tissues of rats given 1 mg/kg/day doses of [^{14}C]-NI-25 (acetamiprid) for 15 days.

Exp. Group	G. I. Tract and Contents	Blood	Liver	Kidney
Group I[a]				
Males	4.08±0.51	0.20±0.02	0.67±0.07	0.12±0.01
Females	3.25±0.46	0.17±0.06	0.66±0.03	0.11±0.01
Group Ii[b]				
Males	2.25±0.35	0.09±0.03	0.32±0.01	0.07±0.02
Females	1.68±0.41	0.08±0.05	0.23±0.14	0.05±0.03
Group Iii[c]				
Males	0.0085±0.0011	0.0049±0.0019	0.0058±0.0009	0.0022±0.0003
Females	0.0096±0.0010	0.0044±0.0025	0.0039±0.0002	0.0017±0.0001
Group Iv[c]				
Males	0.024±0.009	0.000±0.000	0.006±0.004	0.008±0.001
Females	0.034±0.014	0.000±0.000	0.000±0.001	0.004±0.000

[a]1 hr, [b]10 hrs; [c]96 hrs

Data taken from Tables XIII, XIV, XV, and XVI (pp. 54-57), MRID 44988506.

B. PHARMACOKINETIC STUDIES

Time-course for radioactivity in the blood (equivalent to parent compound) following a single oral dose (Groups B, C, and CN-B from MRID 44988505) revealed relatively rapid absorption and clearance. Estimates of kinetic parameters are shown in Table 6. It is clear that absorption was rapid with peak concentrations occurring within 1-2 hours for the low- dose (1 mg/kg) groups and only slightly later (~4 hrs) for the high-dose (50 mg/kg) group. Clearance from the blood appeared to be nearly complete within 48 hours (Table 7). The time course for blood radioactivity was not significantly different for males and females. Additionally, the blood concentration values for Groups B and D (low and high-dose groups, respectively) at similar sampling times reflected the 50-fold difference in dose. Tissue half-lives were also reported for the examined tissues/organs from rats in Groups B (single low dose) and D (single high dose). For Group B, half-lives ranged from 3.5 - 5.9 hours for males and 2.9 - 5.9 hours for females. For the Group D, tissue half-lives ranged from 6.0 - 8.5 hours for males and 6.3-8.3 hours for females. These data suggest that elimination from tissues was not greatly affected by a 50-fold dose increment. Generally, the time course in tissues was similar to that for blood, and there was no evidence for sequestration of administered radioactivity.

TABLE 6. Pharmacokinetic parameters of [^{14}C]-NI-25 (acetamiprid) in rats following a single oral dose (1 or 50 mg/kg/day) (MRID 44988505)

Parameter[a]	Experimental Group					
	B (1 mg/kg)		D (50 mg/kg)		CN-B (1 mg/kg)	
	Males	Females	Males	Females	Males	Females
C_{max} (mg/kg)	0.91	1.01	40.50	31.46	0.97	0.98
t_{cmax}[b] (hrs)	0.5-2.0	0.5-1.0	3.0-5.0	3.0-7.0	1.0	1.0-2.0
$t_{1/2\ abs}$ (hrs)	7.11	5.84	8.07	15.03	5.90	11.29

[a] Mean of five rats
[b] Range for five rats
Data taken from Table 2, p. 29, MRID 44988505.

TABLE 7. Time-course of blood radioactivity in rats following a single oral dose of [^{14}C]-NI-25 (acetamiprid) (MRID 44988505)

Time (hrs)	mg eq. concentration/kg			
	Group B (1 mg/kg)		Group D (50 mg/kg)	
	Males	Females	Males	Females
1	0.771	0.803	31.46	34.77
5	0.458	0.505	–	–
14	–	–	15.45	10.71
10	0.221	0.190	–	–
24	–	–	5.05	5.30
96	0.001	0.001	0.07	0.07

Data taken from Table 5-6, pp. 32-33, MRID 44988505.

Pharmacokinetic parameters derived from the 15-day repeat dose study (MRID 44988506) are summarized in Table 8. Pharmacokinetic indices indicated that absorption ($t_{1/2abs}$) and excretion ($t_{1/2\ elim}$) following oral administration (1 mg/kg/day) was rapid. The t_{cmax} of ~2.8 hours also reflected a relatively rapid absorption process. These data are consistent with the time-course data (i.e., 1, 10, and 96-hour assessments) for tissue radioactivity. There were no biologically significant gender-related differences. The kinetics were similar to those from the single-dose metabolism study (MRID 44988505).

TABLE 8. Pharmacokinetic parameters of [^{14}C]-NI-25 (acetamiprid) in rats following oral administration (1 mg/kg/day) for 15 days[a] (MRID 44988506)

Parameter	Experimental Group V	
	Males	Females
C_{max}	0.798±0.111 µg/mL	0.861±0.132 µg/mL
t_{cmax}	2.80±0.637 hrs	2.81±0.894 hrs
$t_{1/2\ abs}$ (hrs)	1.35±0.825 hrs	1.18±0.868 hrs
$t_{1/2\ elim}$ (hrs)	4.42±1.10 hrs	5.56±1.93 hrs
AUC	8.35±1.12 µg eq. · hr · mL^{-1}	10.3±2.90 µg eq. · hr · mL^{-1}

[a] Animals sacrificed at 48 hours after last dose.
Data taken from Table VIII, p. 46, MRID 44988506.

METABOLITE CHARACTERIZATION STUDIES

NI-25 was extensively metabolized following single intravenous or single oral administration. Metabolites accounted for 79-86% of the administered radioactivity and, in regard to major metabolites, profiles were remarkably similar for males and females and for oral versus intravenous dosing. Only 3-7% of the dose was recovered in the urine and feces as unchanged test article. A metabolism pathway (Figure 1) was proposed by the study authors of the ADME study (MRID 44988505). Urinary and fecal metabolites from the 15-day repeat dose experiment (Group IV of MRID 44988506) were also characterized and reported in MRID44988504.

Plasma – Assessment of metabolites in plasma was not a protocol element of the ADME study (MRID 44988505).

Urine – The major urinary metabolites (those representing 5% or greater of the administered dose and parent compound) from rats given [^{14}C]-NI-25 (acetamiprid) are shown in Table 9. Parent compound represented 7% or less of the administered dose thereby affirming the extensive metabolism of acetamiprid. The most prevalent metabolite resulting from metabolism of the ring-label NI-25 was IC-O (6-chloronicotinic acid) which consistently accounted for 24-28% of the administered radioactivity following dosing with ring-labeled test article. Neither the quantitative nor the qualitative metabolite profiles were affected by dose or gender.

The analysis of urine samples (MRID 44988504) from the repeat-dose experiments (data not reproduced in this Data Evaluation Record) revealed a urinary metabolite profile that was qualitatively and quantitatively similar to that of the single-dose treatments with the exception of slightly increased amounts of the glycine conjugate (IC-O-gly) in the repeat-dose group (10.1% of dose for males and 10.3% of dose for females) versus <4% in the single dose groups.

Feces – The feces represented a minor elimination route for metabolites of acetamiprid (Table 9). Fecal metabolites accounted for only 1-10% of the radioactivity administered orally and 11% of the intravenous dose. The most quantitatively relevant metabolites are listed in Table 9. The fecal metabolite profile for the 15-day repeat-dose groups (MRID 44988504) did not exhibit biologically relevant variations from those of the single-dose groups and affirmed fecal excretion as a minor route of elimination for acetamiprid.

TABLE 9. Metabolite profiles (% of administered radioactivity) in rats following administration of [^{14}C]-NI-25 (acetamiprid)

Matrix/ Metabolite	Group A		Group B		Group D		Group CN-B	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine								
NI-25	3.47	5.22	5.22	4.84	7.15	6.45	3.39	3.93
IM-2-1	12.73	18.29	18.83	18.07	23.84	20.07	16.21	15.87
IC-O	27.74	24.42	27.79	24.90	26.91	26.65	NA[a]	NA[a]
IC-O-gly	3.97	0.61	3.58	1.29	2.82	1.40	NA[a]	NA[a]
Origin-1	7.90	6.20	5.15	4.97	6.73	4.92	NA[a]	NA[a]
Others-1	5.81	3.65	3.04	3.35	2.41	3.05	NA[a]	NA[a]
Others-2	5.62	4.99	7.52	7.55	5.27	4.72	NA[a]	NA[a]
IS-1-1	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	12.88	16.03
IS-2-1	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	34.40	29.33
Others-4	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	7.27	5.53
Feces								
NI-25	0.69	0.91	0.87	0.79	0.60	0.89	0.59	0.59
IM-2-1	0.66	0.69	0.67	0.93	0.64	1.30	0.74	0.69
IC-O	0.39	0.32	0.40	0.62	0.21	0.98	NA[a]	NA
IS-1-1	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	0.27	0.42
IS-2-1	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	1.20	0.90
Others-4	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	NA[a]	0.46	0.54

[a]NA: not analyzed/not applicable.
Data taken from Tables 11 and 12 (pp. 38-39), MRID 44988505.

Bile – No metabolite identification/characterization was performed on bile samples.

Tissues – Assessment of metabolites in plasma was not a protocol element of the ADME studies.

III. DISCUSSION

A. DISCUSSION

Studies were conducted to assess the metabolism and disposition of orally and intravenously administered NI-25 (acetamiprid) in male and female Sprague-Dawley rats. Experiments included single oral or i.v. dose (MRID 44988505) using groups of 5 to 10 rats and doses of 1 or 50 mg/kg of pyridine ring-labeled [^{14}C]-NI-25 (Lot no. EC-09-09, EC-09-10, radiochemical purity 98.9%, chemical purity >99%), and a single 1 mg/kg oral dose group (MRID 44988505) using cyano-labeled [^{14}C]-NI-25 (Lot no. EC-09-21C, radiochemical purity 98.6-99.2%, chemical purity >99%). An additional study

(MRID 44988506) utilized a 15-day repeat-dose protocol in which groups of 3-5 male and female Sprague-Dawley rats were given 1 m/kg/day doses of ring [^{14}C]- NI-25 (Lot No. CFQ8019, radiochemical purity 97%, chemical purity >99%) or non-labeled NI-25 (Lot no. NNI-01, purity >99.9%) and terminated at 1, 10, or 96 hours after the last dose. Vehicle controls received equivalent volumes of 0.9% saline. A biliary excretion study (MRID 44988507) using 4 male and 4 female Sprague-Dawley rats (with saline controls) was conducted using ring-labeled [^{14}C]-NI-25 (Lot no. CFQ8019, radiochemical purity 97.1%, chemical purity >99%) and non-labeled NI-25 (Lot no. NNI-01, purity >99.9%). A summary report (MRID 44988503) provided an overview of the findings of the other reports that assessed the absorption, distribution, metabolism and excretion of NI-25 (acetamiprid). A study to characterize urinary and fecal metabolites (MRID 44988504) utilized biological samples generated by a 1 mg/kg single-dose group reported in MRID 44988506.

There were no treatment-related toxicologic effects, although two rats with bile duct cannulae were intentionally terminated prematurely. Recovery of administered radioactivity for the various experimental groups in the repeat-dose study (MRID 44988506) was 91.7-106% (except Group V which was 71.7-85.% due possibly to loss of urine sample which contained substantial radioactivity), 93-100% for the single-dose study (MRID 44988505),and 89.6-94.5 for the biliary excretion study (MRID 44988507). Overall, these mass balance data are considered acceptable.

Absorption of orally administered NI-25 was rapid and complete based upon urinary excretion data and intravenous administration data. Estimation of absorption by comparison of urinary excretion following intravenous and oral administration (i.e., [urinary excretion oral/urinary excretion, i.v.] x 100) showed that absorption following oral administration was 96-99%. This was consistent with urinary excretion, cage wash, and tissue/body burden data from MRID 44988506, indicating that up to ~65-75% of the administered repeated oral dose was absorbed. There did not appear to be any biologically relevant gender-related differences in any of the groups and total absorption did not vary significantly at the 1, 10, or 96-hour post-treatment sacrifice times in the repeat-dose study.

Urinary excretion was the major route of elimination of [^{14}C]- NI-25 (acetamiprid) associated radioactivity. Excretion of radioactivity following a single oral dose of NI-25 was rapid regardless of dose or label position with the majority (76-97%) of the urinary excretion occurring within 24 hours. Urinary elimination of administered radioactivity was nearly complete within 48 hours. As expected, urinary excretion was somewhat greater for the intravenous dose group than for the oral dose groups but the difference was not biologically relevant or indicative of compromised/saturated absorption processes following oral administration. In the biliary elimination study (MRID 44988507), 24.3% (males) and 36.9% (females) of the administered dose was excreted in the urine.

Consistent with the findings of the single-dose metabolism study (MRID 44988505), results of the repeat dose study (MRID 44988506) also showed urinary excretion to be prevalent. Total urinary excretion did not vary significantly from 1 to 96 hours post

dosing (15-day repeated dose) indicating that most excretion had occurred rapidly (within 24 hours) following each dose. Fecal elimination 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. Fecal excretion of radioactivity by rats in the biliary elimination study was expectedly less; 6.72% (males) and 5.84% (females) than that for the other experimental groups. Biliary elimination data (MRID 44988507) exhibited considerable variability, although mean biliary excretion of radioactivity did not vary notably between genders. Over the 48-hour period, biliary elimination accounted for approximately 19% of the administered radioactivity. Enterohepatic circulation was limited and likely inconsequential.

Pharmacokinetic parameters reflected the rapid absorption and excretion of the NI-25. Peak concentrations occurred within 1-2 hours for the low- dose (1 mg/kg) groups and only slightly later (~4 hrs) for the high-dose (50 mg/kg) group. Clearance from the blood was nearly complete within 48 hours. Tissue half-lives ranged from 3.5 - 5.9 hours for males and 2.9 - 5.9 hours for females in the low-dose group, and 6.0 - 8.5 hours for males and 6.3 - 8.3 hours for females in the high-dose group. These data suggest that elimination from tissues was not greatly affected by a 50-fold dose increment. Consistent with rapid and complete excretion, the time-course in tissues was similar to that for blood. There was no evidence for sequestration of administered radioactivity and no toxicologically significant gender-related differences. Pharmacokinetic parameters derived from the 15-day repeat dose study (MRID 44988506) were similar to those from the single-dose metabolism study. The assessed indices indicated that absorption ($t_{1/2abs}$) and excretion ($t_{1/2\ elim}$) following oral administration (1 mg/kg/day) was rapid. The t_{cmax} of ~2.8 hours also reflected a relatively rapid absorption process. These data are consistent with the time-course data (i.e., 1, 10, and 96-hour assessments) for tissue radioactivity.

Tissue distribution data for the 15-day repeat-dose ADME study (MRID 44988506) revealed that, although radioactivity was widely distributed, tissue burdens represented relatively small amounts (generally <1%) of the administered radioactivity. The greatest radioactivity was expectedly found in the gastrointestinal tract, where up to 3-4% of the administered dose was detected in Group I (single oral 1 mg/kg dose). The greater radioactivity in this tissue could be readily attributed to unabsorbed test material in the lumenal contents which was included in the analysis. Liver and kidney also exhibited somewhat greater levels of radioactivity than did other tissues but did not exceed 0.66% of the dose and declined notably from 1 hour to 96 hours following the last of 15 doses. Again, this is not unexpected due to the metabolic and excretory function of these organs. At 96 hours after the final dose (Groups II and IV, MRID 44988506), radioactivity levels in all tissues generally represented considerably less than 0.001% of the administered dose. There were no significant differences between whole blood radioactivity and plasma radioactivity. No gender-related differences were observed. The data indicate that, 15-day repeat doses of 1 mg/kg do not result in sequestration of the test article or its metabolites.

269

Under the conditions of the reported experiments, NI-25 (acetamiprid) 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 (Group IV of MRID 44988506) 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 (MRID 44988505) that is consistent with available data from the reviewed studies.

These metabolism/kinetics studies (MRID 44988503, 44988504, 44988505, 44988506, and 44988507) in rats are collectively **Acceptable/Guideline** and satisfy the requirements for a Metabolism and Pharmacokinetics Study [OPPTS 870.7485 (§85-1)].

B. STUDY DEFICIENCIES

Low recovery of administered radioactivity in Group V of MRID 44988506 was inadequate, possibly due to loss of urine samples, was not considered to substantively affect the validity of the overall findings.

In the biliary elimination study (MRID 44988507), two rats (one saline control one treated) were terminated prematurely due to loss of cannula patency therefore data are available for only three treated rats of each sex. An assessment of metabolite profiles in bile samples would have provided for a more complete picture of the metabolism of acetamiprid in rats but its absence does not reduce the validity of the collective studies in meeting 85-1 guidelines. Additionally, biliary elimination of the cyano-labeled test article (CN-[^{14}C]NI-25) would have provided additional information regarding possible biliary metabolites.

Identification/characterization of biliary metabolites would have provided a more complete picture of the metabolic fate of acetamiprid in rats. The absence of these data, however, neither invalidate the existing studies nor render them less appropriate for [OPPTS 870.7485 (§85-1)] requirements.

265