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JUL 13 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id # 003125-00414. Imidacloprid. Review of the series 81-8 acute neurotoxicity and 82-7 subchronic neurotoxicity screen studies.

TOX CHEM No.: 497E
PC No.: 129099
Barcode No.: D201204 and D204996
Submission No.: S461935 and S469047

FROM: John Doherty, Ph.D., D.A.B.T. John Doherty 6/14/95
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: Dennis Edwards/Portia Jenkins
Product Manager #19
Registration Division (7508C)

THROUGH: Karl Baetcke, Ph.D. Karl D. Baetcke 7/5/95
Chief, Toxicology Branch I
Health Effects Division (7509C)

I. CONCLUSION

The series 81-8 acute neurotoxicity screen study (MRID No.: 43170301) and 82-7 subchronic neurotoxicity study (MRID No.: 43286401) were reviewed and determined to be ACCEPTABLE and MINIMUM respectively. The acute neurotoxicity study demonstrated a NOEL and LEL of 42 and 151 mg/kg based on decreased activity and tremors. The subchronic study demonstrated a NOEL of 213 mg/kg/day (the highest dose tested) for specific neurotoxicity responses. The systemic NOEL and LEL was demonstrated to be 9.3 and 63.3 mg/kg/day based on decreased body weight gain. No additional series 81-8 and 82-7 acute or subchronic neurotoxicity studies are required at this time.

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II. Background and Action Requested

Miles, Inc has submitted both the series 81-8 (acute) and 82-7 (subchronic) neurotoxicity studies in response to the Agency's previous request for these studies as a part of the conditional registration of imidacloprid. The studies were reviewed and the DERs are attached.

III. Toxicology Branch Comments

The acute neurotoxicity study was determined to have a **NOEL and LEL of 42 and 151 mg/kg** based on decreased motor activity and tremors. The neurotoxicity study was determined to have a LEL of > 3000 ppm (196 in males and 213 mg/kg/day in females) for neurotoxicity. Thus, the LEL in the acute study is **less than** the LEL for subchronic study. It should be noted that administration of the test material was by gavage in the acute neurotoxicity study and the subchronic study adminstration was by the dietary route. The preceding should be considered in the LTL evaluations for imidacloprid.

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IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
81-8. Acute Neurotoxicity Screen -rats Miles, Inc. Study #	Technical Imidacloprid (97.6 to 98.8% batch 2030030	41370301	18/sex Sprague-Dawley rats were dosed by gavage with Imidacloprid (98.8 % pure) at 0, 42, 151 or 307 mg/kg. 6 rats/sex/dose were used for clinical pathology measurements. Twelve rats/sex/dose were assessed for reactions in FOB measurements approximately 90 minutes postdosing and motor activity measurements at approximately 2.5 hours postdosing. Additional FOB and motor activity tests were performed on days 7 and 14. Six rats/sex/dose were examined for neuropathologic lesions. (MRID No.: 43170301). Cage side or FOB assessments effects included tremors (1/12, male and female), and red nasal staining (1/12, male) in the 151 mg/kg dose group. At 307 mg/kg, there were 4 male and 10 female deaths on either day 0 or day 1. The 307 mg/kg dose group exhibited decreased number of rears, grip strength (forelimb and hindlimb), and response to stimuli (auditory, touch, or tail pinch); increases in gait abnormalities and righting reflex impairments and body temperature generally in both sexes. The symptoms regressed by day 5. Motor and locomotor activities were decreased in all dosed females (i.e. 27% in the 42 mg/kg dose group) but were statistically significant at 151 mg/kg (48%, $p < 0.05$) and above. 151 and 307 mg/kg dosed males were decreased on day 0 and were also decreased at 307 mg/kg on days 7 and 14. **The study LOEL is 151 mg/kg based on decreased motor activity and tremors. The NOEL is 42 mg/kg.**	ACCEPTABLE

82-7. Subchronic Neurotoxicity Screen-rat Miles, Inc. Study # 92-472-RF June 13, 1994	Technical Imidaclo-prid (97.6 to 98.8% batch 2030030	43286401	Four groups of 12/sex Fischer strain rats were dosed as control, 150, 1000 or 3000 ppm imidacloprid (technical 98% purity, corresponding to 9.3, 63.3 or 196 in males and 10.5, 69.3 or 213 in females mg/kg/day imidacloprid) for 13 weeks in a subchronic neurotoxicity screen study. 6 additional rats/ sex/dose were also assessed for clinical chemistry and hematology (MRID No.: 43286401). **The LEL for neurotoxicity is > 3000 ppm.** Systemic effects include body weight gain decrease over the first four weeks for the 1000 (22% males, 18% females) and 3000 (50% males, 25% females) ppm dose groups and decreased terminal body weight for both sexes with an associated decrease in forelimb grip strength especially in males. **The LEL for systemic effects is 1000 ppm based on decreased body weight gain and the NOEL is 150 ppm.**	MINIMUM

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Reviewer: John Doherty, Ph.D. 7/12/95
Review Section IV, Toxicology Branch I
Secondary Reviewer: Linnea Hansen, Ph.D. 7/13/95
Toxicology Branch I
Health Effects Division (7509C)

011601

DATA EVALUATION REPORT[1]

STUDY TYPE: Acute oral neurotoxicity screening battery in rats (Guideline series 81-8)

TOX. CHEM. NUMBER: 497E

P.C. CODE: 129099

MRID NUMBER: 43170301, 43285801

TEST MATERIAL: Imidacloprid

SYNONYMS: 1-[(6-Chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine

STUDY NUMBER: 92-412-QR, 94-412-YW

SPONSOR: Miles Inc. Agricultural Division Kansas City, Missouri

TESTING FACILITY: Miles Inc. Agricultural Division, Toxicology Stilwell, Kansas

TITLE OF REPORT: "An acute oral neurotoxicity screening study with technical grade Imidacloprid (NTN 33893) in rats"

AUTHOR: L.P. Sheets and B.F. Hamilton (pathology)

REPORT ISSUED: February 16, 1994 (Supplemental report June 7, 1994)

EXECUTIVE SUMMARY: 18/sex Sprague-Dawley rats were dosed by gavage with Imidacloprid (98.8% pure) at 0, 42, 151 or 307 mg/kg. 6 rats/sex/dose were used for clinical pathology measurements. Twelve rats/sex/dose were assessed for reactions in FOB measurements approximately 90 minutes postdosing and motor activity measurements at approximately 2.5 hours postdosing. Additional FOB and motor activity tests were performed on days 7 and 14. Six

[1]The first draft of this review was prepared by the Clements Inc, Contractor. The original draft was extensively revised by J. Doherty, TB-I HED.

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rats/sex/dose were examined for neuropathologic lesions. (MRID No.: 43170301).

Cage side or FOB assessments effects included tremors (1/12, male and female), and red nasal staining (1/12, male) in the 151 mg/kg dose group. At 307 mg/kg, there were 4 male and 10 female deaths on either day 0 or day 1. The 307 mg/kg dose group exhibited decreased number of rears, grip strength (forelimb and hindlimb), and response to stimuli (auditory, touch, or tail pinch); increases in gait abnormalities and righting reflex impairments and body temperature generally in both sexes. The symptoms regressed by day 5.

Motor and locomotor activities were decreased in all dosed females (i.e. 27% in the 42 mg/kg dose group) but were statistically significant at 151 mg/kg (48%, $p < 0.05$) and above. 151 and 307 mg/kg dosed males were decreased on day 0 and were also decreased at 307 mg/kg on days 7 and 14. **The study LOEL is 151 mg/kg based on decreased motor activity and tremors. The NOEL is 42 mg/kg.**

CLASSIFICATION: CORE GUIDELINE. No significant study deficiencies were noted. The study satisfies the requirement for a series 81-8 acute neurotoxicity screen study.

Special Review Criteria (40 CFR 154.7) None

A. MATERIALS

1. Test Material: NTN 33893 Technical (Imidacloprid)
   Description: Cream-colored powder
   Lot/Batch #: 2030030
   Purity: 98.8% (August 1992); 97.6% (March 1993)
   Stability of compound: 2 years, when frozen
   CAS #: 138261-41-3

2. Vehicle: Imidacloprid was dissolved in 0.5% (w/v) methylcellulose with 0.4% (w/v) Tween 80 in deionized water. Vehicle control animals received an equivalent volume of the vehicle by gavage.

3. Test animals: Rat
   Strain: Sas:CD(SD)BR Sprague Dawley
   Age and weight at study initiation: 9 weeks; males weighed 236-304 g and females weighed 142-185 g; mean body weights of males were 264-267 g and females were 172-176 g (fasted weights)
   Source: Sasco, Inc., St. Louis, Missouri
   Housing: Individually

   Environmental conditions:
   Temperature: 18-26°C
   Humidity: 40-70%
   Air changes: Not reported
   Photoperiod: 12-hour light-dark cycle
   Acclimation period: At least six days prior to group assignment

B. STUDY DESIGN

1. Animal assignment

Animals were weighed and those with body weights which deviated ≥20% of the mean weight were rejected. The remainder of the animals were assigned randomly to the test groups in Table 1 such that mean body weights of each group/sex were similar.

TABLE 1: STUDY DESIGN[a]

Test Group	Dose (mg/kg)	Neurobehavioral Tests/Histopathology[b]	Clinical Pathology
Vehicle	0	12	6
Low	42	12	6
Mid	151	12	6
High	307	12	6

[a]Data from study report, p. 18
[b]Six of the 12 animals/sex/group for neurobehavioral testing were also used for histopathological examinations 15 days after dosing.

Rats were administered test compound by gavage in a total volume of 10 ml/kg. The amount administered per animal was based on the animal's fasted body weight.

Rationale for dose selection: Doses were based on an acute oral study (Miles Report No. 100040, 1989) in which the NOEL for clinical signs was 50 mg/kg in males and 100 mg/kg in females. Signs of toxicity were seen at 100 mg/kg and 250 mg/kg in males and females, respectively, and mortality was seen at 400 mg/kg (both sexes). In a range-finding study, a dose of 200 mg/kg resulted in reduced activity in males and doses of 345 and 417 mg/kg produced a number of neurotoxic signs in both sexes. Signs of toxicity were apparent 2 hours following treatment.

2. Validation of methodology

Data were referenced from studies conducted with untreated and positive controls. These data established the reproducibility of baseline values and the ability of the functional observational battery, motor activity tests, and neuropathological examinations to detect treatment-related changes (see Appendix 1). These data were provided for review with the current study (MRID 42770301).

3. Preparation and analysis of dosing solutions

Imidacloprid was dissolved in 0.5% (w/v) methylcellulose with 0.4% (w/v) Tween 80 in deionized water at dosing levels of 0, 50, 150, and 350 mg/kg. Dosing solutions were analyzed by a liquid chromatography (method of analysis further described in Miles Report No. 99875, 1990) for test material content. Homogeneity analyses were performed on solutions prepared for dosing at 10 and 500 mg/kg.

Results
Homogeneity analysis: Acceptable; the relative standard deviation of the two doses tested (1 and 50 mg/Ml) was reported to be 2.3% and

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5%, respectively.
Stability analysis: Dosing solutions were stable for 8 days at room temperature with no decline in concentrations.
Concentration analysis: Nominal doses of 50, 150, and 350 mg/kg yielded actual concentrations of 42, 151, and 307 mg imidacloprid /kg, respectively. The actual concentrations were 85, 101, and 87.7% of nominal, respectively. Used rats for post-fixation in

4. Diet - Animals were given food (Purina Mills Rodent Lab Chow #5001-4) and received water *ad libitum* except during the overnight fast prior to dosing when only water was available.

5. Statistics - In general, continuous data were analyzed by ANOVA followed by Dunnett's test. FOB data (continuous) used repeated measures ANOVA followed by one-way ANOVA if there was significant interaction between dose group and test week. This was followed by Dunnett's test to determine which groups were different from controls. Categorical FOB data were similarly analyzed using General Linear Modeling and Categorical Modeling procedures (SAS package) and post-hoc analysis using Dunnett's test and an Analysis of Contrasts, respectively. Motor and locomotor activity were analyzed first using a repeated measures analysis of variance followed by analysis of variance and Dunnett's tests. For weeks where there was a significant treatment-by-interval interaction, one-way ANOVA and Dunnett's test were used to determine which groups differed significantly from controls.

Chemical pathology data were evaluated for homogeneity of variance and groups with homogeneous variance were analyzed with ANOVA followed by Dunnett's test. Non-homogeneous data used the non-parametric Kruskal-Wallis test with the Mann-Whitney U-test for pairwise comparison. Chi-square test followed by one-tailed Fisher's Exact test was used for histologic data.

C. METHODS AND RESULTS

1. Observations Cage-side inspections of animals were conducted once daily for signs of *toxicity*, and twice daily (except weekends and holidays, when animals were inspected only once).

Mortality occurred in 4/18 high-dose males and 10/18 high-dose females either on the day of treatment or during the following day. No deaths occurred in other groups or after day 2. Deaths were stated by the study author as being caused by acute cholinergic toxicity consistent with the nicotinic agonist action of imidacloprid.

Table 2 summarizes important clinical observations. The treatment-related clinical signs observed occurred primarily in high-dose males and females on days 0 and 1, and included increased incidence of tremors, incoordinated gait, decreased activity, coolness to touch, and nasal staining. In males at 151 mg/kg, one exhibited tremors and one red nasal staining. Signs in surviving males and females were generally resolved within one to five days

after dosing. Other infrequent signs such as lacrimal staining and labored breathing occurred beginning at 7 days and were not considered treatment-related.

TABLE 2. Incidence of Clinical Signs in Rats Receiving a Single Gavage Dose of Imidacloprid[a]

Parameter	Incidence by Dose Level (mg/kg)							
	0	42	151	307	0	42	151	307
	Males				Females			
Tremors	0	0	1	9	0	0	0	6
Incoordinated gait	0	0	0	8	0	0	0	5
Nasal stain	0	0	2	7	0	0	0	3
Decreased activity	0	0	0	7	0	0	0	5
Coolness to touch	0	0	0	4	0	0	0	4

a. Data extracted from Study No. 92-412-QR, Table 1 (pp. 35-36).
b. There were 12 rats /sex/dose except for 11 in the mid dose male group.

2. Body Weight Animals were weighed 1 week prior to dosing, at initiation, and weekly, thereafter.

Results - No treatment-related effects were observed.

3. Food Consumption Food consumption was not determined.

4. Functional Observational Battery (FOB)

A functional observational battery was conducted on all animals 1 week prior to dosing, at 90 minutes after dosing, and on days 7 and 14. Animals were evaluated without knowledge of the treatment group to which they belonged.

Home cage observations
X Posture*
X Piloerection*
X Gait*
X Muscle fasciculations or tremors*
X Convulsions*
X Vocalization*

Manipulative observations
X Ease of removal from cage*
X Ease of handling*
X Muscle tone*
X Palpebral closure*
X Pupil size*

Open field observations
X Posture*
X Piloerection*
X Gait*
X Muscle fasciculations or tremors*
X Convulsions*
X Respiration*
X Stereotypy*
X Arousal*
X Number of rears*
X Bizarre behavior*
X Urination*
X Defecation*

X Lacrimation*
X Salivation*
X Staining of eyes, nose, mouth*
Fur appearance*
Exophthalmos*

Physiological measures
X Body weight*
X Rectal temperature*

Response observations
X Auditory response*
X Approach response*
X Righting reflex*
X Pupil response*
X Touch response*
X Pain response*

Neuromuscular tests
X Forelimb grip strength*
X Hindlimb grip strength*
X Hindlimb footsplay*

*Recommended by Subdivision F (March 1991) Guidelines

Most FOB parameters were scored as being either present or absent or were scored using graded categories to denote differences from controls. Fecal pellets, urine pools, and number of rears were counted. Fore- and hindlimb grip strength were measured in kilograms using a protocol described only as "SOP E-201." Hindlimb footsplay was measured in mm. Body temperature was obtained using a rectal thermistor. The relationship to treatment was determined by comparison with both pretest and control data. Low incidence findings, not reasonably expected to be observed in controls, were considered to reflect a treatment-related effect.

Results - Table 3 summarizes selected FOB results for day 0. Treatment-related effects in males receiving 151 mg/kg were home-cage observations of decreased activity (sitting or lying in the cage rather than standing, 9/11 males compared with 7 in the controls). In the open field, tremors were seen in one male and one female and nasal staining was observed in one male. These were considered dosing-related although the response was considered minimal but at the next higher dose level these same parameters were pronounced.

At the 307 mg/kg dose, incoordinated gait and decreased activity were observed in the home-cage. In the open field, an increased incidence decreased activity with minimal head or body movement, tremors, incoordinated gait, and decreased rearing were observed in both sexes; a decreased response to touch or tail pinch was observed in several males and females, and the righting reflex was impaired in two male and two female rats (two other female rats could not be tested due to poor condition). Also, there was a significant decrease in mean core body temperature in males and females. Nasal stain was observed in two high-dose males. Auditory stimulus response in five females was impaired, and one female exhibited urine staining. Footsplay (day 0) was not affected by dosing. However, footsplay measurements were not performed on four females because it was determined that they could not position their feet on landing.

Table 4 compares forelimb and hindlimb grip strength measurements on day 0. Both measures were reduced at the high dose. Forelimb grip strength was 12-17% lower than controls in

males and females (non-significant) and hindlimb grip strength was 35% and 17% lower than controls in males and females, respectively (significant in males). Differences in grip strength at lower doses were considered incidental. At days 7 and 14, no biologically important changes were observed.

Other observations were considered incidental and not related to dosing, particularly those occurring after days 2 or 3. These conclusions are based on single occurrences, lack of dose response, lack of consistency between sexes, minimal evidence of impaired performance, and/or an incidence within the range of normal from previous studies. During FOB testing at days 7 and 14, no toxicologically significant effects were evident for home-cage or open field observations, stimuli responses, or footsplay measurements.

5. Motor Activity. Motor activity and locomotor activity were measured in all rats in automated figure-8 mazes (Columbus Instruments) equipped with 8 photobeams located around the perimeter of each apparatus. Motor activity counts (number of photobeam interruptions) and locomotor activity counts (number of photobeam interruptions eliminating consecutive counts for a given beam) were measured in 10-minute intervals over a 90-minute period. Motor activity and locomotor activity were assessed 30 minutes after completion of the FOB. The activity in controls (day 0) was lower than controls at pretest, which is a consistent finding in acute studies and is attributed to the overnight fast prior to dosing (unique to day 0).

Results - Table 5 illustrates data for summary sessions. On the day of dosing, a dose-related decrease in total session motor activity was observed in males receiving 151 (25% decrease, not significant[2]) and 307 (73% decrease, not significant) mg/kg and in females at 42 (25% decrease, not significant), 151 (48% decrease, $p < 0.05$) and 307 (81% decrease, $p < 0.05$) mg/kg imidacloprid. Total session locomotor activity was also decreased to about the same percentage differences but statistical differences were not reported (Refer to Table 5). Results of the 10-minute interval testing for motor and locomotor activity on day zero are presented in Appendices 2 and 3, respectively to illustrate the habituation of the test animals.

On days 7 and 14, decreases (not statistically significant) were still observed in motor and locomotor activity in surviving high-dose males. For example, the high dose was 436 $\pm$ 116 vs 578 $\pm$ 213 counts for the control at day 7 (25% less) and 477 $\pm$ 146 counts vs 626 $\pm$ 262 counts for the control (33% less). The

[2]Study report statistics, not reassessed by HED).

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residual decrease was indicated by the study author as a manifestation of severe acute toxicity rather than neurotoxicity.

6. Clinical Pathology Blood samples for clinical chemistry and hematological analyses were collected from the orbital plexus of the satellite non-fasted animals (6/sex/group) 24 hours after dosing. The CHECKED (X) parameters were examined.

a. Hematology

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

Results - Changes in hematological values (increased hemoglobin and hematocrit, mature neutrophilia and lymphopenia) were attributed to dehydration and stress following exposure to the lethal dose of imidacloprid.

b. Clinical Chemistry

Electrolytes
X Calcium
X Chloride
Magnesium
X Phosphorus
X Potassium
X Sodium
Enzymes
X Alkaline phosphatase
Cholinesterase
X Creatine kinase
X Lactate dehydrogenase
X Serum alanine aminotransferase (also SGPT)
X Serum aspartate aminotransferase (also SGOT)
X Gamma-glutamyl transpeptidase
Glutamate dehydrogenase
Other
X Albumin
X Blood creatinine
X Blood urea nitrogen
X Cholesterol
X Globulins (Haptoglobin)
X Glucose
X Total bilirubin
X Total serum protein
X Triglycerides
X Uric acid

Results - Serum triglycerides were decreased for mid- and high-dose males and females compared to controls. This decrease was the only treatment-related effect seen at a sub-lethal dose. Serum potassium and cholesterol were reduced in high-dose females, and a significant reduction in serum ALT activity was seen in high-dose males and females.

7. Sacrifice and Pathology At termination of the study, 6 rats/sex/dose were anesthetized with sodium pentobarbital and perfused with phosphate-buffered sodium nitrate and then with a solution of 4% (w/v) glutaraldehyde and 4% (w/v) formaldehyde in

phosphate buffer. These animals underwent a complete gross pathological examination and brain weights were obtained. The remaining surviving rats from the main group were sacrificed by carbon dioxide asphyxiation and also were examined grossly.

Tissues collected from the perfused rats for post-fixation in 10% buffered formalin included the entire brain and spinal cord, both eyes (with optic nerves), bilateral peripheral nerves (sciatic, tibial, sural), the gasserian ganglia, and skeletal muscle (gastrocnemius).

> Brain (6 levels - olfactory region, forebrain, midbrain, pons, medulla oblongata, and cerebellum) and spinal cord (cross sections of the cervical, thoracic, and lumbar regions) were embedded in paraffin and sections were stained with hematoxylin and eosin or Luxol Fast Blue/Cresyl violet and Sevier-Munger silver nitrate. Skeletal muscle, dorsal root ganglia, cauda equina, eyes, gasserian ganglia, and tissues from the hippocampus and cerebellar cortex were embedded in glycol methacrylate and sections were stained with Cresyl violet. Peripheral nerves were embedded in Epon and sections were stained with Toluidine blue. Tissues from the control and high-dose animals were examined histologically.

Results - No treatment-related effects were observed at the gross or histopathological examination of tissues from imidacloprid-treated rats.

D. DISCUSSION

This study is classified as CORE GUIDELINE. Based on the above information the LOEL for neurotoxicity was 151 mg/kg and the NOEL was 42 mg/kg.

E. STUDY DEFICIENCIES

— None. No significant deficiencies were noted.

A signed and dated quality assurance statement was present.
A signed and dated GLP statement was present.

APPENDIX 1: Validation of Methods

UNTREATED RATS: STUDY Nos. 90-992-HN and 90-992-IF. Untreated rats examined for motor activity (not fasted) and clinical signs/FOB at single or multiple times (0, 4, 8, 13 weeks) demonstrated acceptable reproducibility of results and provided a historical control data base. Animals demonstrated habituation to mazes during 90-min test sessions and with repeated testing.

CHLORPROMAZINE AND TRIADIMEFON. STUDY NO. 90-912-IL. Rats were tested for motor activity after intraperitoneal administration of 200 mg/kg triadimefon, 2 mg/kg chlorpromazine, 5 Ml saline vehicle/kg, or no treatment. Pronounced increased motor activity was observed in animals treated with triadimefon (about 2X higher; statistically significant) and decreased activity was observed in animals treated with chlorpromazine (about 50% lower; statistically significant only for female early interval measurements) when compared to controls. Motor activity was decreased in all groups on the day of dosing due to fasting.

ACRYLAMIDE. STUDY NO. 91-992-KT. Clinical/FOB examinations and neurohistopathology were performed on rats after intraperitoneal administration of 0, 25, or 50 mg/kg acrylamide in 1 Ml/kg saline. Clinical/functional effects included ataxia, piloerection, muscle fasciculations, tremors, and urine or oral stains (data not analyzed statistically). Statistically significant increases in peripheral neuropathy and axonal degeneration in spinal cord were observed.

TRIPHENYLTIN. STUDY NO. 91-962-LO. Rats were administered 0 or 12 mg/kg triphenyltin intraperitoneally in 1 Ml saline/kg and neural tissues were processed for histopathological examination. Statistically significant increases in the incidence of neuronal necrosis in the olfactory tract, piriform cortex, and hippocampus; chromatolysis of large neuronal soma in the pons, medulla, spinal cord, dorsal root ganglia, and gasserian ganglia; axonal or nerve fiber degeneration in the spinal cord and several peripheral nerves; and digestion chamber in dorsal root ganglia, gasserian ganglia, spinal cord, and sciatic nerve were observed.

CARBARYL. STUDY NO. 91-962-LR. FOB and clinical examinations were performed on rats after intraperitoneal administration of 0, 15, or 30 mg/kg carbaryl in 1 Ml 2% Cremophor EL/kg. Effects observed in treated animals were characteristic of carbamate poisoning and included urine, oral, nasal, and perianal staining, ataxia, decreased touch and approach reactions, repetitive chewing, muscle fasciculations, and tremors. Results were not analyzed statistically.

TABLE 3. Functional Observation Battery Data for Rats Receiving a Single Gavage Dose of Imidacloprid[a,b]

Parameter	Functional Observation Battery Data by Dose Level (mg/kg) 0	42	151	307
	Males			
Sitting or lying —Home cage, day 0	7/12	7/12	9/11	11/12
Tremors —Home cage, day 0	0/12	0/12	0/11	3/12 (1.7)
—Open field, day 0	0/12	0/12	1/11 (1.0)	10/12## (1.6)
Incoordinated gait —Home cage, day 0	0/12	0/12	0/11	2/12 (1.0)
—Open field, day 0	0/12	0/12	0/11	5/12# (1.2)
Nasal stain (red) —Open field, day 0	0/12	0/12	1/11	2/12
Touch response (no reaction) —Open field, day 0	0/12	1/12	0/11	4/12#
Tail pinch (no reaction) —Open field, day 0	0/12	1/12	0/11	3/12
Number of rears (Mean ± S.D.) —Open field, day 0	1.7 ± 1.6	1.9 ± 1.6	1.9 ± 2.5	0.8 ± 1.3
Body temperature (°C) (Mean ± S.D.) —Day 0	37.4 ± 0.4	37.1 ± 0.5	36.7 ± 0.6	35.4 ± 1.5*
	Females			
Sitting or lying —Home cage, day 0	2/12	6/12	4/12	11/12##
Tremors —Home cage, day 0	0/12	0/12	0/12	9/12## (2.0)
—Open field, day 0	0/12	0/12	1/12 (1.0)	11/12## (2.0)
Incoordinated gait —Home cage, day 0	0/12	0/12	0/12	8/12## (1.9)
—Open field, day 0	0/12	0/12	0/12	10/12## (1.9)
Touch response (no reaction) —Open field, day 0	0/12	0/12	0/12	7/12##
Tail pinch (no reaction) —Open field, day 0	0/12	0/12	0/12	9/12##
Number of rears (Mean ± S.D.) —Open field, day 0	4.4 ± 2.5	4.6 ± 3.5	3.6 ± 3.5	0.9 ± 2.9*
Body temperature (°C) (Mean ± S.D.) —Day 0	37.9 ± 0.8	37.7 ± 0.8	37.3 ± 0.4	32.4 ± 2.1*

[a] Data extracted from Study No. 92-412-QR, Table 3 (pp. 41-43 and 53-56).
[b] Numbers in parentheses indicate average severity, where 1 = slight and 2 = moderate-to-severe.

* Significantly different from control, $p \leq 0.05$
\# Significantly different from control, $p \leq 0.05$ using Fisher's exact test performed by the reviewers
\## Significantly different from control, $p \leq 0.01$ using Fisher's exact test performed by the reviewers

TABLE 4. Grip Strength Data for Rats Receiving a Single Gavage Dose of Imidacloprid[a,b]

Parameter	Grip Strength Data by Dose Level (mg/kg)			
	0	42	151	307
Males				
Forelimb grip strength (kg) (Mean ± S.D.) –Day 0	1.02 ± 0.16	1.03 ± 0.11 (101)	0.98 ± 0.17 (96)	0.90 ± 0.17 (88)
Hindlimb grip strength (kg) (Mean ± S.D.) –Day 0	0.43 ± 0.10	0.43 ± 0.10 (100)	0.43 ± 0.11 (100)	0.28 ± 0.09* (65)
Females				
Forelimb grip strength (kg) (Mean ± S.D.) –Day 0	0.87 ± 0.14	0.80 ± 0.16 (92)	0.83 ± 0.15 (95)	0.72 ± 0.20 (83)
Hindlimb grip strength (kg) (Mean ± S.D.) –Day 0	0.30 ± 0.08	0.31 ± 0.11 (103)	0.34 ± 0.08 (113)	0.25 ± 0.07 (83)

[a] Data extracted from Study No. 91-412-QR, Table 4 (pp. 64 and 68).
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p \leq 0.05$

TABLE 5. Overall Motor and Locomotor Activity Data for Rats Receiving a Single Gavage Dose of Imidacloprid[a,b]

Parameter	Motor Activity Data by Dose Level (mg/kg) 0	42	151	307
		Males		
Motor Activity (day 0)	318 ± 133	302 ± 106 (95)	237 ± 77 (75)	87 ± 50 (27)
Locomotor Activity (day 0)	116 ± 34	105 ± 29 (91)	92 ± 34 (79)	26 ± 23 (22)
		Females		
Motor Activity (day 0)	504 ± 262	366 ± 194 (73)	263 ± 93* (52)	96 ± 71* (19)
Locomotor Activity (day 0)	166 ± 84	124 ± 46 (75)	89 ± 11 (54)	18 ± 30 (11)

[a] Data extracted from Study No. 92-412-QR, Tables 6 and 7 (pp. 73-76).
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p \leq 0.05$

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APPENDIX 2. Interval Motor Activity Data on Day 0 for Rats Receiving a Single Gavage Dose of Imidacloprid[a,b]

Parameter	Motor Activity Data by Dose Level (mg/kg) 0	42	151	307
		Males		
Interval 1	151 ± 65	152 ± 46 (101)	105 ± 35 (70)	35 ± 23* (23)
Interval 2	84 ± 29	74 ± 37 (88)	48 ± 31* (57)	12 ± 14* (14)
Interval 3	38 ± 35	30 ± 17 (79)	25 ± 11 (66)	9 ± 11* (24)
Interval 4	23 ± 25	4 ± 5* (17)	15 ± 15 (65)	8 ± 12 (35)
Interval 5	8 ± 12	9 ± 16	16 ± 16	6 ± 8
Interval 6	8 ± 14	12 ± 17	9 ± 9	3 ± 6
Interval 7	5 ± 11	7 ± 15	4 ± 7	7 ± 9
Interval 8	3 ± 6	2 ± 3	8 ± 16	5 ± 5
Interval 9	0 ± 0	13 ± 24	8 ± 14	3 ± 5
		Females		
Interval 1	160 ± 48	139 ± 53 (87)	122 ± 35 (76)	27 ± 26* (17)
Interval 2	96 ± 41	70 ± 48 (73)	49 ± 14* (51)	6 ± 8* (6)
Interval 3	68 ± 41	49 ± 34 (72)	22 ± 19* (32)	12 ± 16* (18)
Interval 4	40 ± 45	30 ± 26 (75)	20 ± 19 (50)	10 ± 14 (25)
Interval 5	33 ± 32	22 ± 27	13 ± 15	4 ± 8*
Interval 6	32 ± 34	16 ± 18	11 ± 14	3 ± 7*
Interval 7	35 ± 38	11 ± 12*	11 ± 18*	4 ± 6*
Interval 8	23 ± 35	14 ± 19	4 ± 9	13 ± 17
Interval 9	18 ± 32	16 ± 19	10 ± 12	16 ± 18

[a] Data extracted from Study No. 92-412-QR, Table 8 (pp. 78 and 82).
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p \leq 0.05$

APPENDIX 3. Interval Locomotor Activity Data on Day 0 for Rats Receiving a Single Gavage Dose of Imidacloprid[a,b]

Parameter	Locomotor Activity Data by Dose Level (mg/kg)			
	0	42	151	307
	Males			
Interval 1	65 ± 17	59 ± 13 (91)	46 ± 15* (71)	16 ± 14* (25)
Interval 2	27 ± 10	27 ± 11 (100)	20 ± 13 (74)	3 ± 4* (11)
Interval 3	12 ± 11	9 ± 5 (75)	8 ± 4 (67)	2 ± 3* (17)
Interval 4	7 ± 8	1 ± 2* (14)	5 ± 6 (71)	0 ± 1* (0)
Interval 5	2 ± 3	2 ± 4	5 ± 6	1 ± 3
Interval 6	2 ± 4	3 ± 4	2 ± 3	0 ± 0
Interval 7	1 ± 2	1 ± 3	1 ± 2	1 ± 1
Interval 8	0 ± 1	0 ± 1	2 ± 3	1 ± 2
Interval 9	0 ± 0	2 ± 5	2 ± 4	0 ± 1
	Females			
Interval 1	60 ± 17	57 ± 1 (95)	50 ± 7 (83)	9 ± 14* (15)
Interval 2	35 ± 15	25 ± 13 (71)	17 ± 3* (49)	2 ± 5* (6)
Interval 3	20 ± 13	15 ± 10 (75)	6 ± 4* (30)	2 ± 4* (10)
Interval 4	11 ± 11	8 ± 8 (73)	5 ± 3 (45)	2 ± 3* (18)
Interval 5	9 ± 10	7 ± 9	5 ± 6	0 ± 1
Interval 6	12 ± 15	3 ± 6	2 ± 3*	1 ± 2*
Interval 7	11 ± 13	3 ± 4*	2 ± 3*	0 ± 0*
Interval 8	6 ± 10	2 ± 4	1 ± 2	1 ± 2
Interval 9	3 ± 6	4 ± 7	2 ± 4	2 ± 3

[a] Data extracted from Study No. 92-412-QR, Table 9 (pp. 86 and 90).
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p \leq 0.05$

Reviewed by: John Doherty, Ph.D., D.A.B.T.
Section IV, Toxicology Branch I (7509C)
Secondary reviewer: Linnea Hansen, Ph.D. 6/9/95
Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 82-7. Subchronic neurotoxicity screen--rats.

MRID NO.: 432864-01 TOX. CHEM. NO.: 497E
PC No.: 129099

TEST MATERIAL: Technical Imidacloprid (97.6% to 98.8% purity, batch 2030030.

STUDY NUMBER: 92-472-RF

SPONSOR: Miles Incorporated.

TESTING FACILITY: Miles Inc., Stilwell, Kansas

TITLE OF REPORT: "A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Imidacloprid (NTN 33893) in Fischer 344 Rats.

AUTHORS: Larry P. Sheets and B.F. Hamilton (pathology)

REPORT ISSUED: June 13, 1994
[In-life phrase: Approximately January 25, 1993 to April 30 1993]

EXECUTIVE SUMMARY:

Four groups of 12/sex Fischer strain rats were dosed as control, 150, 1000 or 3000 ppm imidacloprid (technical 98% purity, corresponding to 9.3, 63.3 or 196 in males and 10.5, 69.3 or 213 in females mg/kg/day imidacloprid) for 13 weeks in a subchronic neurotoxicity screen study. 6 additional rats/sex/dose were also assessed for clinical chemistry and hematology (MRID No.: 43286401).

The LEL for neurotoxicity is > 3000 ppm.

Systemic effects include body weight gain decrease over the first four weeks for the 1000 (22% males, 18% females) and 3000 (50% males, 25% females) ppm dose groups and decreased terminal body weight for both sexes with an associated decrease in forelimb grip strength especially in males. **The LEL for systemic effects is 1000 ppm based on decreased body weight gain and the NOEL is 150 ppm.**

Classification: MINIMUM. The study did not demonstrate a LEL for neurotoxicity. The study satisfies the guideline requirement for a series 82-7 subchronic neurotoxicity screen study in rodents.

Quality Assurance Statement: Provided.
Good Laboratory Practice Statement: Provided

Statement of Data Confidentiality: Provided. No claim of confidentiality.

REVIEW

Experimental Constants:

[illegible] in males at week [illegible] all values were 2-3 times higher [illegible] control was [illegible] and treated groups were [illegible]

Test Chemical:

Chemical:	Imidacloprid (NTN 33893 Technical)
Chem Name:	1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine[1]
Purity:	97.6 to 98.8%
Batch No.:	2030030 Research Sample Stock, 816255903 Bayer Production Number.
Appearance:	Cream colored powder
Cas No.:	138261-41-3
Pharmacological Class:	Agonist of the alpha-bungarotoxin sensitive nicotine ACh receptor

Analytical Chemistry: [The Analytical Chemistry report is in Appendix III of the study report and was prepared by K.D. Moore.]

Test substance analysis. The identity and concentration of the technical grade of imidacloprid was confirmed by NMR and mass spectrometry. The concentration was assessed to be 98.8, 97.6 and 98.6% for three samplings of material (batch number 2030030/816255903).

Homogeneity. Samples of 50 and 4000 ppm were assessed at the top, middle and bottom (3 samples/layer). The means for all nine samples were 46.2 with coefficient of variation of 6.5% and 3671 with a coefficient of variation of 7% for the 50 and 4000 ppm diets respectively. This indicated acceptable homogeneity although the percentages were 7.6% and 9% lower than nominal.

Stability. Data were presented that indicated almost no breakdown of imidacloprid in 50 and 4000 ppm diets when stored for 14 days at room temperature or for 28 days at -23° C. Treated diets were not used after 1 week.

Concentration of actual diets. The means of diets assessed on weeks 1,4,5,6,9 and 14 indicated 140 ± 5, 963 ± 78 and 3027 ± 61 ppm which is considered sufficiently close to the nominal concentrations of 150, 1000 and 3000 ppm.

Test System:

Species;	Rat
Strain:	Fischer 344 CDF(F-344)/BR
Source:	Sasco, Inc. Madison, WI.
Age:	8 weeks at start of treatment.
Randomization:	Weight based computer assisted.
Diet:	Purina Rodent Lab Chow 5001-4
Vehicles:	Acetone (evaporated after mixing) and 1% corn oil.
Acclimation:	One week
Housing:	Individually

Statistics: The study report asserts that the following statistical tests were performed. In general the statistics were conducted using the SAS or the INSTEM Computer systems. Bartlett's test for homogeneity of data was tested at 0.001 and

[1]Structure not available in HED computer Chem Structure Library as of January 1995.

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all other tests were at 0.05 level of significance.

Statistical Test	Parameter Investigated
Bartlett's test followed by ANOVA (repeated measures and one way) followed by Dunnett's test if positive. If Bartlett's test [illegible] positive, the Kruskal-Wallis tests. followed by a Mann Whitney U test was used.	Continuous data [illegible] sciatic fibers, tibia squina, [illegible] nerves, gastrocnemius muscle and
General Linear Modeling and Categorical Modeling (CATMOD) Procedures with post-hoc comparisons using Dunnett's test and an Analysis of Contrasts	Categorical data collected from the FOB
Repeated measures ANOVA followed by a one-way ANOVA if there was a significant interaction with test occasion. Followed by Dunnett's test. ..... Two-way Repeated-Measures ANOVA, using both test interval and test occasion as the repeated measures, followed by a Repeated Measures ANOVA to determine on which weeks there was a significant treatment by interval interaction. Followed by a one way ANOVA to determine at which intervals there was a significant treatment effect. This was again followed by Dunnett's test to determine which groups were significantly different from the control group.	Motor activity/session data ..... Motor activity/interval data.
Chi-Square test followed by a one-tailed Fisher's Exact Test	Ophthalmology and micropathology.

Specific Methods and Results

Basic Experimental Design:

Four groups of 18 rats/sex were dosed in their diet with 0, 150, 1000 or 3000 ppm of imidacloprid for 90 days. 12/sex were used for FOB and motor activity assessment and six of these were eventually prepared for neuropathology assessment. The remaining 6/sex were used for clinical chemistry and hematology determinations during weeks 4 and 13 of exposure.

The selection of the test dose levels was based on earlier subchronic studies in which dose levels of 2400 and 3000 produced 13-21% decrease in body weight and hepatotoxicity. No evidence of clinical signs indicative of neurotoxicity were reported in the preliminary studies.

1. Deaths and Clinical Signs. Assessed twice daily for survival and clinical signs and a detailed examination once weekly. All rats survived to termination and there were no treatment related clinical signs based on cage side observations.

2. Body Weight and Gain. Table 1 below illustrates body weight gain, terminal body weight and compound consumption.

Table 1. Body weight gain and terminal body weight and compound consumption.

Dose Level	Males			Females		
	Wt gain[1]	Term BW[2]	Compound[3]	Wt. gain.	Term BW	Compound
Control	62.6	301.8±10	----	25.7	167.7±9	----
150 ppm	63.4	303.9±13	9.3±0.1	23.0 (11%)	165.7±7 (1%)	10.5±0.1
1000 ppm	49.1 (22%)	279.1±15* (7.5%)	63.3±0.9	21.0 (18%)	162.5±5* (3%)	69.3±0.7
3000 ppm	31.4 (50%)	253.6±10* (16%)	196±2	19.4 (25%)	155.1±5* (7.5%)	213±2

1. Body weight gain for first 4 weeks (difference between day 28 and day 0). Data are from Table 2 of study report but calculation of body weight gain was by this reviewer and not analyzed statistically. Number in () is the percent decrease in body weight gain.
2. Terminal body weight in grams. Data from table 2 of study report. The number in () is the percent decrease relative to controls.
3. Compound consumed in mg/kg/day. Date from table 4 of study report.
* $p < 0.05$ ANOVA and Dunnett's test.

Both the mid and high dose groups were noted to have an initial (i.e. first four weeks) decrease in body weight and terminal body weight was lower for both sexes. The 11% decrease in body weight gain for the low dose female group is noted by TB-I but is not considered a definite effect level. Terminal body weight averages for the mid dose males and females were approximately 8% and 3% lower and for the high dose groups were 16% and to 8% (as calculated by this reviewer) for males and females. Corresponding food consumption was decreased ($p < 0.05$) for both the mid (for most weeks, i.e about 10% for males and 8% for females)and high (for all weeks, i.e. about 20% for males and 13% for females) dose groups for both sexes.

3. Functional Observational Battery. Based on Table 5 of the study report, the following observations were made pretreatment, weeks 4, 8 and 13.

A. Qualitative Observations.

Observations in Home Cage:
posture
piloerection

Removal from Home Cage or Handling:
ease of removal
reaction to handling

gait abnormalities
involuntary motor-clonic
involuntary motor-tonic
vocalizations
(other)

muscle tone
palpebral closure
pupil size
pupil response
lacrimation
salivation
stains
other

Open field observations
piloerection
respiratory abnormalities
posture
involuntary motor-clonic
involuntary motor-tonic
stereotypy
bizarre behavior
gait abnormalities
vocalizations
arousal
rearing counts
defecation (# of fecal boli)
urination (# of urine pools)

Reflex/physiological observations
approach response
touch response
auditory response
tail pinch response
righting reflex
body temperature

Quantitative Observations:
forelimb grip strength
hindlimb grip strength
landing foot splay

Of all of these parameters, the only parameter reported affected by treatment was forelimb grip strength. Table 2 below depicts these data together with body weight data for males at each interval and for females at week 8 only.

Table 2. Forelimb grip strength and body weight correlation in males.

Dose Level	Males				Females
	Pretest	Week 4	Week 8	Week 13	Week 8
Control	135/0.39 (346)	214/0.72 (297)	261/.94 (278)	301/.98 (307)	161/.60 (268)
150 ppm	137/0.38 (358)	216/0.70 (309)	264/0.87 (303)	304/.98 (310)	156/.69 (226)
1000 ppm	136/0.39 (349)	206/0.72 (286)	**240*/0.82 (293)**	**280*/.96 (292)**	**152*/.68 (224)**
3000 ppm	136/0.43 (316)	**189*/0.6 (300)**	**217*/0.72* (301)**	**255*/.89 (287)**	**145*/.61 (238)**

1. Data are mean body weight (the std deviations were about 5% for both sexes)/forelimb grip strength (kg, the std deviaticns were about 18% for males and 20% for females). The number in () is the ratio of the body weight/grip strength. Data are from Table 6 of the study report (pages 64-71).

The study author asserts that the NOEL and LEL for an effect on forelimb grip strength are 150 and 1000 ppm but recognizes that the decrease in grip strength correlates with the decrease in body weight. On only one occasion (high dose group at week 8) the forelimb grip strength decreased to a statistically significant degree but at this occasion, the ratio

of body weight to grip strength was still equivalent with the lower dose and control values.

TB-I considers that since a constant ratio between body weight and grip strength was maintained, that the apparent effect on grip strength was not an effect of imidacloprid on the physiology of strength (i.e. neurotoxicity). In addition, there were no associated changes in FOB parameters that would be expected if the test material was actually affecting forelimb grip strength (i.e hindlimb grip strength, coordination, lethargy etc.). The decrease in forelimb grip strength is concluded by TB-I to be related to the decrease in body weight.

In conclusion, TB-I concludes that there are no direct effects on FOB parameters as caused by imidacloprid demonstrated in this study.

5. Motor Activity. Motor activity was assessed in figure 8 mazes (Columbus Instruments) capable of detecting both increases and decreases in activity. Each session consisted of 90 minutes with 10 minute intervals and was conducted with low light and white noise. Motor activity was measured as the number of interruptions of infrared light beams that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity during the test session.

The study author asserts that there were no compound related effects on motor activity. Table 8 (photocopied from the study report) indicates that there were no statistical differences in the mean total counts for the 90 minute sessions for females at any of the four assessment times. The high dose group, however, was consistently higher (i.e. about 30%) in total count. The standard deviations, however, were largest also for this group. Figure 6 (photocopied form the study report) depicts the individual interval counts for each dose level for both males and females at week 4. Although the high dose group appears higher for some intervals for males, the habituation rate was the same. The mid dose group appears to have several sessions with lower counts that the control. Similar plots were obtained for the pretest, week 8 and week 13 motor activity assessments.

TB-I concurs with the study author that there is no demonstrated effect of imidacloprid on motor activity in this study.

6. Ophthalmology No effects of treatment were noted on pupillary reflex (pen light test) or following mydriasis and slit lamp examination of the conjunctiva, cornea and lens. The vitreous humor, retina, choroid and optic disc were further examined using an indirect ophthalmoscope and a condensing lens and no treatment related effects were noted.

7. Clinical Chemistry and Hematology. Assessed at weeks 4 and 13. {These assessments are not required in a series 82-7 study}. The following parameters were indicated as being affected by the study author but the author also asserts that these changes are of no known toxicological significance.

-creatine kinase: In males at week 4, all values were 2-3 times higher than the control i.e. control was 11±4 and treated groups were 27 ± 11 U/l to 34 ± 6 and at week 13 the control was 265 ± 95 U/l and the mid (129 ± 43 or -51%) and high (49 ± 10 or -82%) dose showed statistical significant decrease. In females, the mid (99 ± 23 U/l, -47%) and high (107 ± 31, -43%) dose groups were lower than the control 187 ± 37 U/l.

-triglycerides: high dose females (-40% at week 4 and -35% at week 13) and high dose males (-36% at week 4, -26% at week 13) were reduced.

-lactate dehydrogenase: mid and high dose females and high dose males were reduced.

-Total protein and albumin: reduced in high dose females (both -6% at week 4 and -5% and -8% at week 13).

-Phosphate: high dose males (-8 to -9% at weeks 4 and 13) and females (-9% at week 4) were reduced.

TB-I notes, however, that there is no recognized interpretation of decreases in creatinine kinase and lactate dehydrogenase. The data on creatinine kinase varied widely from week 4 to week 13 (i.e. the control was 24 times higher) making the reported differences questionable. As for total protein, triglycerides and phosphate reported differences, TB-I does not note any covariants that would indicate that these parameters are actually being affected by the test material to a toxicologically significant level. The reported results of these parameters being affected by imidacloprid should be compared with the series 82-1 (subchronic feeding) and 83-5 (chronic feeding/carcinogenicity study) with this chemical. For example, the latter three may be associated with the liver changes induced by imidacloprid but there were no liver pathology slides prepared for this study.

8. Gross pathology. Six rats/sex from each group were prepared for perfusion and following perfusion given a gross necropsy exam. No compound related lesions were noted at gross necropsy. Brain weight was assessed following removal from the skull. Absolute brain weight was not reportedly affected by treatment. Relative brain weight for males was increased by 10 and 15% for the mid and high dose groups, This was considered to be consistent with the lower body weight.

9. Histopathology and Perfusion Studies. Perfusion was performed under anesthesia (pentobarbital) and perfused by the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation with Universal fixative (4% w/v glutaraldehyde and 4% w/v EM grade formaldehyde) in phosphate buffer. The following sections were prepared.

-Coronal sections from six levels of the brain:

olfactory region, forebrain, midbrain, pons, medulla (oblongata, and cerebellum).

-Cross- and longitudinal- sections from three levels of the spinal cord: cervical, thoracic and lumbar.

The above were embedded in paraffin and stained with H&E.

-Dorsal root ganglia (dorsal and ventral fibers), cauda equina, gasserian ganglion, eyes, optic nerves, gastrocnemius muscle and additional tissue from the hippocampus and cerebellar cortex.

The above were embedded in glycol methacrylate and sectioned at 2 - 3 microns and stained using Lee's stain.

-Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in epoxy resin, cut at approximately 1 micron and stained with toluidine blue.

-Additional sections from the brain and spinal cord were stained with Luxol Fast Blue/Cresyl Violet and Sevier-Munger stains.

The pathology report was prepared by B.F. Hamilton. The control and high dose group rats were assessed microscopically. Dr. Hamilton's conclusion was that there were no compound related effects noted. Since there were no indications of neuropathology in the high dose group, the low and mid dose groups were not assessed microscopically. TB-I concurs with Dr. Hamilton's report.

E. Immunochemistry: No immunochemistry assessments for GFAP were made.

STUDY CONCLUSION: This study is classified as CORE MINIMUM. The limiting factor is that the high dose did not demonstrate neurotoxicity per se as is expected for a chemical tested in a series 82-7 subchronic neurotoxicity study. Since imidacloprid is a ACh receptor agonist some signs of neurotoxicity would have been expected. The study was otherwise consistent with the current guideline recommendations. The study demonstrated systemic toxicity (reduced body weight gain in both sexes) and is considered to have been assessed at adequate otherwise doses.

Since the forelimb grip strength decrease correlated closely with body weight and because there were no other covariants that also indicated neurotoxicity, the change in grip strength is not regarded as an indication of neurotoxicity per se. TB-I does not consider that this study demonstrates any indications of neurotoxicity responses to imidacloprid.

TB-I did not include the clinical chemistry findings in its overall assessment of the systemic effects of imidacloprid. Of the parameters affected, creatine kinase and lactate

dehydrogenase, were shown to be decreased but there is no recognized toxicological significance for decreases in these parameters. The other three parameters (phosphate, triglycerides and protein) are considered only possible responses to treatment that would be best assessed subchronic and in the chronic feeding studies. In this study there were also no associated pathology (pathology on non-neuronal tissues were not provided) or other covariants that were affected to indicate that the apparent changes in these parameters were other than incidental changes. The results of the clinical chemistry analysis do not affect the NOEL/LEL for this study.

Note: An appended table entitled "Positive Controls for Neurotoxicity Studies Miles Laboratories" provides references for positive controls to demonstrate the validation of the assay methods used.

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APPENDIX 1: Validation of Methods

UNTREATED RATS: STUDY Nos. 90-992-HN and 90-992-IF. Untreated rats examined for motor activity (not fasted) and clinical signs/FOB at single or multiple times (0, 4, 8, 13 weeks) demonstrated acceptable reproducibility of results and provided a historical control data base. Animals demonstrated habituation to mazes during 90-min test sessions and with repeated testing.

CHLORPROMAZINE AND TRIADIMEFON. STUDY NO. 90-912-IL. Rats were tested for motor activity after intraperitoneal administration of 200 mg/kg triadimefon, 2 mg/kg chlorpromazine, 5 Ml saline vehicle/kg, or no treatment. Pronounced increased motor activity was observed in animals treated with triadimefon (about 2X higher; statistically significant) and decreased activity was observed in animals treated with chlorpromazine (about 50% lower; statistically significant only for female early interval measurements) when compared to controls. Motor activity was decreased in all groups on the day of dosing due to fasting.

ACRYLAMIDE. STUDY NO. 91-992-KT. Clinical/FOB examinations and neurohistopathology were performed on rats after intraperitoneal administration of 0, 25, or 50 mg/kg acrylamide in 1 Ml/kg saline. Clinical/functional effects included ataxia, piloerection, muscle fasciculations, tremors, and urine or oral stains (data not analyzed statistically). Statistically significant increases in peripheral neuropathy and axonal degeneration in spinal cord were observed.

TRIPHENYLTIN. STUDY NO. 91-962-LO. Rats were administered 0 or 12 mg/kg triphenyltin intraperitoneally in 1 Ml saline/kg and neural tissues were processed for histopathological examination. Statistically significant increases in the incidence of neuronal necrosis in the olfactory tract, piriform cortex, and hippocampus; chromatolysis of large neuronal soma in the pons, medulla, spinal cord, dorsal root ganglia, and gasserian ganglia; axonal or nerve fiber degeneration in the spinal cord and several peripheral nerves; and digestion chamber in dorsal root ganglia, gasserian ganglia, spinal cord, and sciatic nerve were observed.

CARBARYL. STUDY NO. 91-962-LR. FOB and clinical examinations were performed on rats after intraperitoneal administration of 0, 15, or 30 mg/kg carbaryl in 1 Ml 2% Cremophor EL/kg. Effects observed in treated animals were characteristic of carbamate poisoning and included urine, oral, nasal, and perianal staining, ataxia, decreased touch and approach reactions, repetitive chewing, muscle fasciculations, and tremors. Results were not analyzed statistically.

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