9-3-93

CASWELL FILE

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

010537

SEP 3 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: I.D. Nos.: 003125-UEE, 003125-UEG, 3F04169, 3H05655. Imidacloprid. Evaluation of Toxicity Data Submitted and Identification of Outstanding Toxicology Data Requirements

| | |
|---|---|
| Tox. Chem. No. | 497E |
| PC Code No. | 129099 |
| DP Barcode Nos. | D186041, D189035, D187142, D187173 |
| Submission Nos. | S432450, S436791, S430201, S430226 |

From: Myron S. Ottley, Ph.D.
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To: Portia Jenkins/Dennis Edwards, Jr. (PM19)
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Through: Marion P. Copley, D.V.M., D.A.B.T.
Section Head
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Through: Karl Baetcke, Ph.D.
Branch Chief
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Health Effects Division (H7509C) 8/29/93

## I. CONCLUSIONS

The existing database supports the following uses for the following formulations: Registration — NC-Food/Feed Use Confidor 2 Flowable
Registration — NC-Food/Feed Use Confidor 2.5% Granular
Tolerance Petition — F Petition for Apples, cotton
Tolerance Petition — I Pet-Food Additive " "

Recycled/Recyclable
Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

## II. ACTION REQUESTED

TB-1 received for evaluation the several studies required to fulfill data requirements for registration of NTN 33893 for food use. These data were submitted by Bayer AG and/or Miles, Inc.

## III. RESULTS/DISCUSSION

The requirements (CFR 158.135) for Food Use for the Technical are listed below in Table 1.

**Table 1.**

| Test | | Technical | | Formulations§ | |
|---|---|---|---|---|---|
| | | Required | Satisfied | Required | Satisfied |
| 81-1 | Acute Oral Toxicity | Y | Y | Y | Y |
| 81-2 | Acute Dermal Toxicity | Y | Y | Y | Y |
| 81-3 | Acute Inhalation Toxicity | Y | Y | Y | Y |
| 81-4 | Primary Eye Irritation | Y | Y | Y | Y |
| 81-5 | Primary Dermal Irritation | Y | Y | Y | Y |
| 81-6 | Dermal Sensitization | Y | Y | Y | Y |
| 81-7 | Acute Delayed Neurotox. (Hen) | N | - | N | - |
| 82-1 | Oral Subchronic (Rodent) | Y | Y | N | - |
| 82-1 | Oral Subchronic (Non-Rodent) | Y | Y | N | - |
| 82-2 | 21-Day Dermal | Y | Y | N | - |
| 82-3 | 90-Day Dermal | N[1] | - | N | - |
| 82-4 | 90-Day Inhalation | N[2] | Y[4] | N | - |
| 82-5 | 90-Day Neurotoxicity (hen) | N[3] | - | N | - |
| 82-6 | 90-Day Neurotoxicity (mammal) | N[3] | - | N | - |
| 83-1 | Chronic Toxicity (Rodent) | Y | Y | N | - |
| 83-1 | Chronic Toxicity (Non-rodent) | Y | Y | N | - |
| 83-2 | Oncogenicity (Rat) | Y | Y | N | - |
| 83-2 | Oncogenicity (Mouse) | Y | Y | N | - |
| 83-3 | Developmental Toxicity (one species) | Y | Y | N | - |
| 83-3 | Developmental Toxicity (two species--rodent & non-rodent) | Y | Y | N | - |
| 83-4 | Reproduction | Y | Y | N | - |
| 83-5 | Chronic/Oncogenicity | Y | Y | N | - |
| 84-2 | Mutagenicity—Gene Mutation | Y | Y | N | - |
| 84-2 | Mutagenicity—Structural Chromosomal Aberrations | Y | Y | N | - |
| 84-4 | Mutagenicity—Other Genotoxic Effects | Y | Y | N | - |
| 85-1 | General Metabolism | Y | Y | N | - |
| 85-2 | Dermal Penetration | N | - | N | - |

| Test | Technical | | Formulations§ | |
|---|---|---|---|---|
| | Required | Satisfied | Required | Satisfied |
| 86-1 Domestic Animal Safety | N | - | N | - |
| Special Studies for Ocular Effects | | | | |
| Acute Oral (Rat) | N | - | N | - |
| Subchronic Oral (Rat) | N | - | N | - |
| Six-month Oral (Dog) | N | - | N | - |

Legend Y = yes N = no § Confidor 2 Flowable (24.1%) and Confidor 2.5% Granular Formulations

1 Not required based on lack of dermal toxicity observed in the 21-day dermal study, and based on expected exposure.

2 Not required since significant exposure via inhalation not expected.

3 Not required since no evidence of neurotoxicity observed in acute or chronic exposure.

4 Although not required in this case, TB-1 received from the Submitter and reviewed a subchronic inhalation study (MRID 422730-01) and classified it as Core Minimum, acceptable for regulatory purposes.

5 DER attached

## A. ACUTE TOXICITY

The Acute toxicity data on the Technical is summarized below in Table 2A. Tables 2B and 2C summarize the acute toxicity data on the 24.1% and 2.5% Formulations, respectively.

TABLE 2A. SUMMARY OF ACUTE TOXICITY OF NTN 33893 TECHNICAL

| TEST | RESULTS | CATEGORY |
|---|---|---|
| 81-1<br>Acute Oral Toxicity—Rats<br>Study No.: T 2033060<br>Date: December 15, 1989<br>MRID No.:420553-31 | LD50: Males: 424 mg/kg<br>Females: >450 to <475 mg/kg<br>Study is Acceptable<br><br>Toxic Signs: Apathy, labored or transient labored breathing, transient accelerated breathing, decreased motility, transient staggering gait, blepharophimosis, transient trembling and transient spasms. | II |
| 81-2<br>Acute Dermal Toxicity—Rabbit<br>Study No.: T 5033063<br>Date: November 15, 1989<br>MRID No.: 420553-32 | LD50: >5000 mg/kg (Limit Test)<br>Study is Acceptable<br><br>Toxic Signs: decreased body weight gain in females | IV |
| 81-3<br>Acute Inhalation Toxicity—Rat<br>Study Nos.: T 2025951<br>T 3025952,<br>T 4025953<br>Date: June 6, 1988<br>MRID No.: 420553-33 | LC50: ♂ & ♀: One 4-hr dose: >5.323 mg/L<br>Five 6-hr doses: >0.505 mg/L<br><br>Study is Acceptable<br><br>Toxic Signs: Single exposure → Difficult breathing, reduced motility, piloerection, slight tremors, decreased body weight gains<br>Repeated exposure → transient decrease in female body wt. gain, dark spleen & isolated foci, increase microsomal enzyme induction. | III |
| 81-4<br>Primary Eye Irritation—Rabbit<br>Study No.: T 8025515<br>Date: February 15, 1988<br>MRID No.: 420553-34 | Primary Irritation Index: 0.0<br>Study is Acceptable<br><br>Toxic Signs: Minimal redness and/or swelling of conjunctivae, clearing within 24 hours. | IV |
| 81-5<br>Primary Dermal Irritation—Rabbit<br>Study No.: T 8025515<br>Date: February 25, 1988<br>MRID No.: 420553-35 | PIS: 0.0 (non-irritating)<br>Study is Acceptable<br><br>Toxic Signs: Slight erythema. | IV |
| 81-6<br>Dermal Sensitization—Guinea Pig<br>Study No.: T 9025651<br>Date: March 15, 1988<br>MRID No.: 420553-36 | Not a Sensitizer<br>Study is Acceptable<br><br>Toxic Signs: None except in positive controls | - - |

TABLE 2B. SUMMARY OF ACUTE TOXICITY OF NTN 33893 24.1% FLOWABLE FORMULATION

| TEST | RESULTS | CATEGORY |
|---|---|---|
| 81-1<br>Acute Oral/Rat<br>Study No. 89-012-DV<br>Date: Feb. 26, 1990<br>MRID No.: 422563-13 | LD50 Male >4870 mg/kg<br>Female 4143 mg/kg (calculated<br><br>Study is Acceptable<br><br>Toxic Signs: Tremors, Decreased activity, lacrimation with stain, convulsions, oral discharge and stain, urine stain, alopecia, decreased body weight gain. | III |
| 81-2<br>Acute Dermal/Rat<br>Study No. 89-025-EB<br>Date:Feb. 22, 1990<br>MRID No.: 422563-15 | LD50 >2000 mg/kg<br><br>NOEL (local and systemic): <2000 mg/kg (Limit Test)<br>LOEL (local and systemic: 2000 mg/kg<br><br>Study is Acceptable<br><br>Toxic Signs: Transient erythema and muscle fasciculation, decreased body weight gain in males. | III |
| 81-3<br>Acute Inhalation/Rat<br>Study No. 89-042-EG<br>Date: Feb. 27, 1990<br>MRID No.: 422563-17 | LC50 >5330 mg/m3<br><br>NOEL <5060 mg/m3<br>LOEL 5060 mg/m3<br><br>Study is Acceptable<br><br>Toxic Signs: Death, hypoactivity, dyspnea, lethargy, tremors, decreased body weight gain in males. | IV |
| 81-4<br>Eye Irrit./Rabbit<br>Study No.:89-335-DZ<br>Date: Jan. 15, 1990<br>MRID No.: 422563-19 | TIS: TIME 1hr 24hr 48hr 72hr 7d 14d<br>IRRIT. SCORE 1.0 0.3 0.2 0.0 0 0<br><br>Study is Acceptable<br><br>Toxic Signs: Conjunctival redness and discharge, chemosis. | III |
| 81-5<br>Primary Dermal Irritation/Rabbit<br>Study No. 89-325-DU<br>Date: Jan. 15, 1990<br>MRID No.: 422563-21 | PIS: 0.0 Non-irritating.<br><br>Study is Acceptable<br><br>Toxic Signs: None | IV |
| 81-6<br>Dermal Sensitization/Guinea pig<br>Study No. 89-324-DO<br>Date: Feb. 22, 1990<br>MRID NO. 422563-23 | Conclusion: Not a Sensitizer<br><br>Study is Acceptable<br><br>Toxic Signs: None except in positive controls. | N/A |

TABLE 2C. SUMMARY OF ACUTE TOXICITY OF NTN 33893 2.5% GRANULAR FORMULATION

| TEST | RESULTS | CATEGORY |
|---|---|---|
| 81-1<br>Acute Oral/Rat<br>Study No. 89-012-DY<br>Date: Feb. 26, 1990<br>MRID No.: 422563-24 | LD50 Male. >4820 mg/kg (5000 mg/kg nominal, Limit Test)<br><br>Study is Acceptable<br><br>Toxic Signs: Increased body weight. No other signs | IV |
| 81-2<br>Acute Dermal/Rat<br>Study No. 89-025-DS<br>Date: Jan. 15, 1990<br>MRID No.: 422563-25 | LD50 >2000 mg/kg (Limit Test)<br><br>Study is Acceptable<br><br>Toxic Signs: None. | III |
| 81-3<br>Acute Inhalation/Rat<br>Study No. 89-042-DX<br>Date: Feb. 26, 1990<br>MRID No.: 422563-26 | LC50 >5092 mg/m3 (95% Confidence Intervals)<br><br>Study is Acceptable<br><br>Toxic Signs: None. | IV |
| 81-4<br>Eye Irrit./Rabbit<br>Study No.:89-335-DT<br>Date: Jan. 15, 1990<br>MRID No.: 422563-27 | TIS: TIME 1hr 24hr 48hr 72hr 7d 14d<br>IRRIT. SCORE 2.3 1.2 1.0 0.5 0.2 0.0<br><br>Study is Acceptable<br><br>Toxic Signs: conjunctival redness, chemosis and discharge | III |
| 81-5<br>Primary Dermal<br>Irritation/Rabbit<br>Study No. 89-325-ED<br>Date: Jan. 15, 1990<br>MRID No.: 422563-28 | PIS: 0.0 Non-irritating.<br><br>Study is Acceptable<br><br>Toxic Signs: None | IV |
| 81-6<br>Dermal<br>Sensitization/Guinea pig<br>Study No. 89-324-DN<br>Date: Feb. 22, 1990<br>MRID NO. 422563-29 | Conclusion: Not a Sensitizer<br><br>Study is Acceptable<br><br>Toxic Signs: None except in positive controls. | N/A |

## B. SUBCHRONIC TOXICITY

Two studies were reviewed: a) a 21-day dermal in rabbits (82-2; MRID 422563-29) and b) a 90-day inhalation in rats (82-4; MRID 422730-01). Each study is classified as Core-Minimum or Core-Guideline.

In the 21-day dermal study, New Zealand white rabbits (5 male and 5 female/group) were exposed to NTN Technical 6 hr/d, 5 d/wk for four weeks at 1,000 mg/kg/d, the limit dose. No dermal or systemic effects of toxicological importance were observed. Based on these results the dermal and systemic NOEL is 1000 mg/kg/day, and the dermal and systemic LOEL is >1000 mg/kg/day.

In the 90-day inhalation study, groups of 10 male and 10 female Wistar rats were exposed (nose only) to analytical concentrations of 0.006, 0.031 or 0.191 mg/L, for 6hr/d, 5 d/wk for 4 weeks. No toxicological effects were observed. Based on these results, the NOEL is 0.191 mg/L and the LOEL is >0.191 mg/L.

## C. CHRONIC/ONCOGENIC TOXICITY

Three chronic feeding and/or carcinogenicity studies are available: a) Chronic Study in Dogs (83-1b; MRID: 422730-02); b) Two-Year Feeding/Oncogenicity Study in Rats (83-1, 83-2; MRID: 422563-31 and 422563-32); and c) Two-Year Oncogenicity Study in Mice (83-2; MRID: 422563-35, 422563-36).

In the chronic dog study, groups of 4 male and 4 female Beagle dogs were fed NTN Technical in the diet daily for 52 weeks, and examined for signs of toxicity. Dose levels were 0, 200, 500 and 1250 ppm (average intake was 0, 6.1, 15.0 and 41.0 mg/kg/d). The high dose was increased to 2500 ppm (72 mg/kg/d) from Week 17 onwards due to lack of toxicity at 1250 ppm. A transient decrease in food consumption, probably due to palatability, was observed during Weeks 1,2, 17, & 18 (males), and at Weeks 2 and 17 - 20 (females) at 1250/2500 ppm. Increased plasma cholesterol and liver cytochrome P-450 levels were seen at 2500 ppm. It was concluded that the NOEL is 1250 ppm, with a LOEL of 2500 ppm.

In two chronic/onco rat study (two studies reviewed as one since dose levels were complementary), groups of 50 male and 50 female Bor WISW(SPF Cpb) rats were administered NTN Technical in the diet for 24 months at 0, 100, 300 and 900 ppm, and at 0 and 1800 ppm and examined for signs of toxicity and carcinogenicity. The results are as follows:

Chronic Effects: NOEL: 100 ppm (5.7 mg/kg/d in ♂, 7.6 mg/kg/d in ♀)
LOEL: 300 ppm. Increased thyroid lesions in ♂ at 300 ppm and above and in ♀ at 900 ppm (73.0 mg/kg/d) and above; decreased body weight gain in females at 300 ppm (24.9 mg/kg/d) and above; weight changes in liver, kidney, lung, heart, spleen, adrenals, brain and gonads in

♂ and/or ♀ at 900 ppm (51.3 mg/kg/d in ♂, 73.0 mg/kg/d in ♀) or 1800 ppm.

Oncogenicity: No apparent treatment-related effect.

In the mouse onco study, (two studies reviewed as one since dose levels were complementary), groups of 60 male and 60 female B6C3F1 mice were fed daily doses of NTN 33893 Technical in the diet at 0, 100, 330, 1000 ppm and 0, 2000 ppm and examined for signs of toxicity and carcinogenicity. The results are as follows:

Chronic Effects: NOEL: 1000 ppm (208 mg/kg/d in ♂, 274 mg/kg/d in ♀)
LOEL: 2000 ppm, based on decreased body weight, decreased food consumption and decreased water intake, in both sexes.

Oncogenicity: No apparent treatment-related effect.

## D. DEVELOPMENTAL TOXICITY

Two studies are available: a) Developmental toxicity in rats (83-3a; MRID 422563-38), and b) Developmental toxicity in rabbits (83-3b; MRID 422563-39).

In the rat study, NTN 33893 Technical was administered by gavage to HSD(SD) rats at 0, 10, 30, and 100 mg/kg/d during Gestational Days 6 - 16. The Maternal NOEL = < 10 mg/kg/d; the LOEL = 10 mg/kg/d, based on decreased body weight gain. At 100 mg/kg/d, Decreased food consumption was observed. The Developmental NOEL = 30 mg/kg/d; the LOEL = 100 mg/kg/d, based on increased wavy ribs.

In the rabbit study, NTN 33893 Technical was administered by gavage to HSD(SD) rats at 0, 10, 30, and 100 mg/kg/d during Gestational Days 6 - 16. The Maternal NOEL = 24 mg/kg/d; the LOEL = 72 mg/kg/d, based on decreased body weight, increased resorption, increased abortion, and death. The Developmental NOEL = 24 mg/kg/d; the LOEL = 72 mg/kg/d, based on decreased body weight, increased skeletal abnormalities.

## E. REPRODUCTIVE TOXICITY

One study is available, (83-4; MRID 422563-40) and is classified as Core Minimum.

In this study, Wistar/Han rats were fed NTN 33893 Technical in the diet during the mating, pregnancy, lactation and post-weaning periods at 0, 100, 250, or 700 ppm. (0, 100, 250 ppm and 700 ppm during premating.) The Parental NOEL = 700 ppm (≈55 mg/kg/d); the LOEL >700 ppm. The Reproductive NOEL = 100 ppm (≈ 8 mg/kg/d); the LOEL = 250 ppm (≈ 19 mg/kg/d) based on decreased pup body weight in both generations.

## F. MUTAGENICITY

Several mutagenicity studies are available. They are summarized on Table 3 below. Data requirements for these FIFRA TOX. Guidelines are satisfied by these submissions; no further studies need be submitted at this time.

Table 3.

| Study Type (MRID No.) | Title (Report No.) | Reported Results | TE Evaluation |
|---|---|---|---|
| Gene mutation-Ames (422563-41) | "NTN 33893 Reverse Mutation Assay (Salmonella typhimurium and Escherichia coli)," Report No. 101276 | Negative for inducing reverse mutation in bacteria exposed to doses up to 5000 ug/plate. | ACCEPTABLE |
| Gene mutation-mamm. cell (422563-42) | "NTN 33893 Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay in Vitro," Report No. 098584 | Negative for inducing forward mutation in CHO (mammalian) cells treated up to 1222 ug/ml | ACCEPTABLE |
| Gene mutation-Ames (422563-43) | "NTN 33893 Salmonella/Microsome Test to Evaluate for Point Mutagenic Effects," Report No. 098570 | Negative up to 12,500 ug/plate | ACCEPTABLE |
| Chromosome Ab. in vivo (422563-44) | "NTN 33893 In Vivo Cytogenetic Study of the Bone Marrow In Chinese Hamster to Evaluate for Induced Clastogenic Effects" Report No. 100021 | Negative for chromosome breakage up to 2000 ug/ml | ACCEPTABLE |
| Chromosome Ab. in vitro (422563-45) | "NTN 33893 In Vitro Cytogenetic Study with Human Lymphocytes for the Detection of Induced Clastogenic Effects," Report No. 099262 | Positive at 500 ug/ml -S9 and 1300 ug/ml +S9, both toxic doses | ACCEPTABLE |
| SCE in vivo (422563-46) | "NTN 33893 Sister Chromatid Exchange in Bone Marrow of Chinese Hamster in Vivo," Report No. 099257 | Negative up to 2000 ug/ml | ACCEPTABLE |
| Chromosome Ab.-Mouse MT (422563-47) | "NTN 33893 Micronucleus Test on the Mouse to Evaluate for Clastogenic Effects." Report No. 102652 | Negative, but only tested up to 80 mg/kg | UNACCEPTABLE (report not required at this time) |
| Chromosome Ab. in vivo (422563-48) | "Mouse Germ-Cell Cytogenetic Assay with NTN 33893," Report No. 102654 | Negative, but only tested up to 80 mg/ml | UNACCEPTABLE (but not required at this time) |
| Other genotoxicity (422563-49) | "Clastogenic Evaluation of NTN 33893 in an In Vitro Cytogenetic Assay Measuring Sister Chromatid Exchange in Chinese Hamster Ovary (CHO) Cells," Report No. 102655 | Positive at 500 ug/ml -S9 and 2000 ug/ml +S9, both toxic doses | ACCEPTABLE |
| Other genotoxicity (472563-50) | "Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells," Report No. 099678 | Negative at toxic doses of 400 ug/ml/-S9, 1250 ug/ml/+S9 | ACCEPTABLE |
| DNA repair (411563-51) | "NTN 33893 Rec-assay with Spores in the Bacterial System" Report No. 101275 | Negative up to 5000 ug | ACCEPTABLE |
| DNA repair (422563-52) | "Mutagenicity Test on NTN 33893 In the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay," Report No. 098573 | Negative up to 750 ug/ml, a toxic dose | ACCEPTABLE |
| Other genotoxicity (422563-53) | "NTN 33893 Test on S. Cerevisiae D7 to Evaluate for Induction of Mitotic Recombination," Report No. 102653 | Negative for crossing-over in yeast up to 10,000 ug | ACCEPTABLE |
| Gene mutation-Ames (422563-63) | "WAK 3839 Reverse Mutation Assay (Salmonella typhimurium and Escherichia coli)," Report No. 100668 | Negative up to 5500 ug/plate | ACCEPTABLE |
| Gene mutation-mamm. cell (422563-64) | "WAK 3839 Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HGPRT Assay In Vitro," Report No. 100662 | Negative up to 2000 ug/ml | ACCEPTABLE |
| Gene mutation-mamm. cell (422563-65) | "WAK 3839 Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay In Vitro," Report No. 100661 | Negative up to 2000 ug/ml | ACCEPTABLE |

| Study Type (MRID No.) | Title (Report No.) | Reported Results | TB Evaluation |
|---|---|---|---|
| Chromosome Ab.- Mouse MT (422563-66) | "WAK 3839 or NTN 37571 Micronucleus Test on the Mouse After Intraperitoneal Injection," Report No. 10064 | Negative up to (toxic) 50 mg/kg (ip) | ACCEPTABLE |
| Chromosome Ab.- Mouse MT (422563-67) | "NTN 37571 Micronucleus Test on the Mice after I.P. Treatment," Report No. 100679 | Negative up to (toxic) 80 mg/kg (ip) a non-toxic dose. | UNACCEPTABLE (not required at this time) |
| Chromosome Ab.- Mouse MT (422563-68) | "WAK 3839 Micronucleus Test on the Mouse After Oral Application," Report No. 100663 | Negative up to 100 mg/kg (oral), a non-toxic dose | UNACCEPTABLE |
| Chromosome Ab.- Mouse MT (422563-69) | "NTN 37571 Micronucleus Test on the Mice After Oral Treatment Pilot Study," Report No. 100680 | Negative up to oral 160 mg/kg, toxic dose | ACCEPTABLE |
| Chromosome Ab.- in vitro (422563.70) | "Chromosome Aberration Assay in Chinese Hamster V79 Cells In Vitro with WAK 38391," Report No. 100666 | Negative up to 1000 ug/ml | ACCEPTABLE |
| Chromosome Ab.- in vitro (422563-71) | "NTN 37571 In Vitro Cytogenetic Assay Measuring Chromosome Aberrations in CHO-K1 Cells," Report No. 100678 | Negative up to 1000 ug/ml | ACCEPTABLE |
| DNA repair (422563-72) | "Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats In Vitro with WAK 3839," Report No. 100665 | Negative up to 1333 ug/ml | ACCEPTABLE |



## G. METABOLISM

The metabolism of NTN 33893 in rats was reported in seven studies (85-1), and found to be Core Minimum. They are: a) Methylene- [$^{14}$C] Imidacloprid: Metabolism Part of the General Metabolism Study in the Rat (MRID 422563-54); b) [$^{14}$C]-NTN 33893. Biokinetic Part of the General Metabolism Study in the Rat (MRID 422563-56); c) [Imiazolidine-4,5-$^{14}$C] Imidacloprid: Investigation of the Biokinetic Behavior and Metabolism in the Rat (MRID 422563-57); d) Imidacloprid - WAK 3839: Comparison of Biokinetic Behavior and Metabolism in the Rat Following Single oral Dosage and Investigation of the Metabolism after Chronic Feeding of Imidacloprid to Rats and Mice (MRID 422563-73); e) A Liquid Chromatographic Method for the Determination of NTN 33893 in Aqueous Dose Mixtures (MRID 422563-59); f) A Liquid Chromatographic Method for the Determination of NTN 33893 in Inhalation Chamber Atmospheres (MRID 422563-58); g) [$^{14}$C]-NTN 33893: Investigations on the Distribution of the Total Radioactivity in the Rat by Whole-body Autoradiography (MRID 422563-55).

These data show that Imidacloprid was rapidly absorbed and eliminated in the excreta (90% of the dose within 24 hours), demonstrating no biologically significant differences between sexes, dose levels, or route of administration. Elimination was mainly renal (70-80% of the dose) and fecal (17-25%). The major part of the fecal activity originated in the bile. Total body accumulation after 48 hr consisted of 0.5% of the radioactivity with the liver, kidney, lung, skin and plasma being the major sites of accumulation. Therefore,

bioaccumulation of Imidacloprid is low in rats. Maximum plasma concentration was reached between 1.1 and 2.5 hr. Two major routes of biotransformation were proposed for Imidacloprid. The first route included an oxidative cleavage of the parent compound rendering 6-chloronicotinic acid and its glycine conjugate. Dechlorination of this metabolite formed the 6-hydroxynicotinic acid and its mercapturic acid derivative. The second route included the hydroxylation followed by elimination of water of the parent compound rendering NTN 35884.

A comparison between [methylene-$^{14}$C]-Imidacloprid and [imidazolidine-4,5-$^{14}$C]-Imidacloprid showed that while the rate of excretion was similar, the renal portion was higher with the imidazolidine-labeled compound. In addition, accumulation in tissues was generally higher with the imidazolidine-labeled compound.

A comparison between Imidacloprid and one of its metabolites, WAK 3839, showed that the total elimination was the same for both compounds. The proposed metabolic pathways for these two compounds were different. WAK 3839 was formed following pretreatment (repeated dosing) of Imidacloprid.

## H. NEUROTOXICITY

Since Imidacloprid is not an organophosphate, the following studies are not required: a) Acute Delayed Neurotoxicity in the Hen (81-7); b) Acute Neurotoxicity Screening Battery in the Rat (81-8-SS); c) 90 Day Neurotoxicity Screening Battery in the Rat (82-7). The Submitter has announced their intention to perform the 90-Day test (82-7) anyway, and has received TB-1 approval on the protocol.

## IV. OTHER TOXICOLOGICAL CONSIDERATIONS

This chemical has been determined to be a Group E Carcinogen (cf. memo from 4/22/92 HED RfD Review Committee, attached).

The Metabolism Committee (see attached memo) has concluded that none of the metabolites of NTN 33893 is of toxicological concern at this time.

## V. REFERENCE DOSE

On April 22, 1993, the HED Reference Dose (RfD) Peer Review Committee recommended that the RfD for NTN 33893 (Imidacloprid) be established at 0.057 mg/kg/d. This value was based on the systemic NOEL of 100 ppm (5.7 mg/kg/day) from the 24-month rat chronic/onco study (MRID 422563-31, 422563-32) and an uncertainty factor (UF) of 100. This RfD has not yet been confirmed by the Agency RfD Work Group.

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

013537

JUN 25 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**Subject:** METABOLISM COMMITTEE MEETING HELD ON JUNE 22, 1993: METABOLISM OF IMIDACLOPRID.

**From:** Francis D. Griffith, Jr., Chemist
Chemistry Branch I - Tolerance Support
Health Effects Division (H-7509C)

**Thru:** Debra F. Edwards, Ph.D., Chief
Chemistry Branch I - Tolerance Support
Health Effects Division (H-7509C)

**To:** The Metabolism Committee
Health Effects Division (H-7509C)

Questions discussed were as follows:

1. Are any additional imidacloprid plant and animal metabolism studies needed at this time?,

2. Do residues of the guanidine and nitrosimino imidacloprid metabolites at the levels reported in the metabolism studies become toxicologically significant?, Is the level of the nitrosimino compound at the levels reported in the technical material toxicologically significant?,

3. Are any of the other imidacloprid metabolites at the levels reported of special toxicological concern?, and

4. Is there any scientific objection to the tolerance expression being for imidacloprid and its metabolites containing the 6-chloropyridinyl moiety?

**INDIVIDUALS IN ATTENDANCE**

METABOLISM COMMITTEE: (signatures indicate concurrence unless otherwise stated)

Karl Baetcke

Richard Lorenger

Michael Metzger

010537

Alberto Protzel

George Ghali

SCIENTISTS: Non-committee members responsible for the data presentation (signatures indicate technical accuracy of the report)

Francis Griffith, Jr.

Robert Quick

Marion Copley

M. Ottley

P. Chin

METABOLISM COMMITTEE MEMBERS IN ABSENTIA: Committee members who were unable to attend the discussions (signatures indicate concurrence with the overall conclusions of the committee)

Richard Schmitt

Reto Engler

Out of the office for 3 weeks RDS 6/25/93

MATERIAL REVIEWED:

The Committee reviewed the CBTS and TOX written presentations which included a discussion of the plant and animal (rat, ruminant, and poultry) metabolism studies showing how the charts of identified metabolites and recovery data for each metabolite fitted the proposed metabolic pathways.

CONCLUSIONS:

1. No additional plant or animal imidacloprid metabolism studies are needed at this time.

2. Residues of the guanidine and nitrosimino imidacloprid metabolites at the levels reported in the plant and animal metabolism studies are not toxicologically significant. These metabolites do not warrant separate regulation.

3. Levels of the nitrosimino imidacloprid at levels less then 40 ppm in the technical mixture need not be removed from the technical material prior to the tech. material being formulated into Confidor. The compound, when at less then 40 ppm, need not be listed on the Confidential Statement of Formula.

4. None of the other imidacloprid metabolites at the levels given are of toxicological concern at this time. No additional metabolism or toxicological studies are needed for any of the other imidacloprid metabolites. None warrant separate regulation.

5. There is no scientific objection to the tolerance expression being in terms of imidacloprid and its metabolites that contain the 6-chloropyridinyl moiety. The residue analytical method measures imidacloprid and its metabolites as 6-chloronicotinic acid (a common moiety method).

cc: Reviewer(FDG),R.Schmitt,R.Engler,A.Protzel,G.Ghali,K.Baetcke,R.Loranger, M.Metzger,M.Copley,P.Chin,M.Ottley,Metabolism Committee File,PP#3F4169,R.F.,Circu,D.Edwards(PM-19,RD),NanGray(CCB/HED).

H-7509C:CBTS:CM#2:Rm804Q:Reviewer(FDG):305-5826:6/22/93:edit:fdg:6/23/93.

RDI:SecHd:RSQuick:6/23/93:BrSrSci:RALoranger:6/23/93.

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

010537

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imidacloprid; Highlights of the RfD/Peer Review Meeting

CASRN: 105827-78-9
EPA Chem. Code: 129059

FROM: George Z. Ghali, PhD G. Ghali 4.29.93
Manager, RfD/Quality Assurance Peer Review Committee
Health Effects Division (H7509C)

TO: James Kariya
Science Analysis Branch
Health Effects Division (H7509C)

The Health Effects RfD/Quality Assurance Peer Review Committee met on April 22, 1993 to discuss and evaluate the toxicology data submitted in support of Imidacloprid registration and to assess the Reference Dose for this chemical.

The RfD/Peer Review Committee recommended that an RfD should be established based upon a NOEL of 5.7 mg/kg/day for increased thyroid lesions observed in males at 16.9 mg/kg/day in a chronic toxicity study in rats. An uncertainty factor (UF) of 100 was recommended to account for the inter-species extrapolation and intra-species variability. On this basis the RfD was calculated to be 0.057 mg/kg/day.

The chemical was classified on the basis of its carcinogenic potential as a "Group E ". No referral to other committees was recommended, and the data available for review did not warrant acute toxicity concern.

Please note that a full RfD/Peer Review Committee report will follow soon.

CC: William Burnam
Kerry Dearfield
Penny Fenner/Crisp
Richard Schmitt
Karl Baetcke
Marion Copley

___I.A. REFERENCE DOSE FOR CHRONIC ORAL EXPOSURE (RfD)

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Chemical -- Imidacloprid
CASRN -- 105827-78-9
On-Line:

___I.A.1. ORAL RfD SUMMARY

Critical Dose -- 5.7 mg/kg-day
UF -- 100
MF -- 1
RfD -- 5.7E-2 mg/kg-day

***Principal Study 1***

Critical Effect -- Increase thyroid lesions in males

Study Type -- 2-Year Rat Feeding/Oncogenicity Study

Reference -- Miles Corp., 1989

NOAEL -- 100 ppm
NOAEL(ADJ) -- 5.7 mg/kg-day (Male)

LOAEL -- 300 ppm
LOAEL(ADJ) -- 16.9 mg/kg-day (Male)

Conversion Factors and Assumptions -- Actual dose tested

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Miles Corp., 1989. MRID No. 42256331, 42256332
Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

Dose levels for the 2 year study were based on results of two subchronic studies, a 98-day feeding study (Miles Corp., 1988) and a 96-day feeding study (Miles Corp., 1989). The 98-day and 96-day studies were conducted using dose levels of 0, 150, 600, and 2400 ppm (0, 7.5, 30, and 120 mg/kg-day) and 0, 120, 600, and 3000 ppm (0, 6, 30, and 150 mg/kg-day), respectively.

Doses up to 150 ppm were tolerated without adverse effects. Slightly retarded growth was observed in both males (up to 8%) and females (up to 10%) at 600 ppm in both studies. Body weight were depressed by up to 14% in males and 16% in females at 2400 ppm and by up to 15% in males and 20% in females at 3000 ppm. Actual data on body weight gains were not presented or statistically analyzed. However, the data show that at 600 ppm body weight gains decrease in males by 7.9% and 9.4% and in females by 3.4% and 16.1% in the two studies, respectively. At 2400 and 3000 ppm body weight gains were depressed in males by 21.7% and 25.7% and in females by 20.5% and 23.4%, respectively.

Also at 2400 and 3000 ppm, elevated phosphatase was observed in males and females and hepatic cell necrosis were observed in males. At 3000 ppm degenerative testicular alterations were observed in males.

In the 2 year feeding/oncogenicity study, groups of Bor WISW (SPF Cpb) rats (50/sex/dose) were administered Imidacloprid at in the diet at dose levels of 0, 100, 300, and 900 ppm (Male: 0, 5.7, 16.9, and 51.3 mg/kg-day;

Female: 0, 7.6, 24.9, and 73) for two years. Since the 900 ppm dose level failed to show adequate toxicity, a companion 2-year study using a single dose level of 1800 ppm (Male: 102.6 mg/kg-day; Female: 143.7 mg/kg-day) was conducted. Animals received food and water ad libitum.

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In the 900 ppm group, body weights of males and females were significantly ($p<0.05$ and/or $p<0.01$) less than their control group (up to 5% in males and up 8% in females) during Weeks 1-4, 9-13, 18 and 22. At 1800 ppm, male and female body weights were significantly less than their control group ($p<0.01$), up to 12% in males and up to 11% in females throughout the study. In the low dose groups, body weight gain were similar to controls. Similarly, body weight gain was at least 10% less than controls only in females at 900 and 1800 ppm.

No toxicological significant changes in clinical chemistry were observed. In the 1800 ppm group, males showed at a persistent decrease (46 to 76%, $p<0.01$) in urine protein concentration when compared with controls. While decrease up to 85% were seen in females, these changes were not statistically significant. No other effects on urinalysis were observed at this dose level or lower.

At 900 ppm, male and female absolute liver weight were decreased 14% ($p<0.05$) at 12 months, and female absolute kidney weights were decreased 13% ($p<0.05$). Female relative brain weights were increased 12% ($p<0.01$) at 12 months. Statistically significant organ weight changes (absolute and relative) were also observed in liver, kidney, brain and heart at 24 months, but were slight (less than 10%) and generally occurred in only one sex. At 1800 ppm, male absolute spleen weights were decreased 17% ($p<0.01$) at 12 months. In femalem, absolute spleen weights were decreased 14% ($p<0.01$) at 24 months, and decreases in female liver (17%), spleen (14%), heart (15%), kidney (11%) and adrenals (17%) were observed ($p<0.01$) at 24 months. Relative testis weight was increased 13% ($p<0.05$) at 12 months and relative ovarian weight was increased 21% ($p<0.01$) at 24 months. No other significant changes in organ weights were observed at lower dose levels.

At 100 ppm, mineralized particles were observed in the colloid of isolated follicles of 12 of 50 male thyroid glands ($p<0.01$) at the 24 month sacrifice. At 300 ppm these thyroid lesions occurred in 31 of 50 males ($p<0.001$) at 24 months. At 900 ppm thyroid lesions were observed in 10 of 10 males ($p<0.05$) at the 12 month sacrifice and 44 of 50 males ($p<0.001$) and 38 of 50 females ($p<0.01$) at the 24 month sacrifice. At 1800 ppm, the thyroid lesions were observed in 10 of 10 males ($p<0.05$) at the 12 month sacrifice and 46 or 50 males ($p<0.001$) and 38 of 50 females ($p<0.001$) at the 24 month sacrifice. In addition, at 1800 ppm there was a 100% decrease in colloid aggregation in male thyroids at 12 months ($p<0.05$) and a 51% decrease at 24 months ($p<0.001$) and in female thyroids (68%, $p<0.01$) at 24 months. Other treatment-related observations at 1800 ppm at 24 months included retinal atrophy (44% increase in females, $p<0.05$), porphyrin accumulation in Harderian glands (65% increase in females, $p<0.05$) and nephropathy - marked reduction in males (65%, $p<0.01$) and females (92%, $p<0.01$).

Based on the increased thyroid lesions in males, the LEL for systemic toxicity in males is 300 ppm (Male: 16.9 mg/kg-day). The NOEL for systemic toxicity in males is 100 ppm (Male: 5.7 mg/kg-day). Based on numerous effects observed at 900 and 1800 ppm, the LEL for systemic toxicity in females is 900 ppm (73 mg/kg-day). The NOEL for systemic toxicity in females is 300 ppm (24.9 mg/kg-day).

Reviewed by: Myron S. Ottley, Ph.D. 6/4/93 010537
Section IV, Tox Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (H7509C) Marion P. Copley 7/21/93

# DATA EVALUATION REPORT

| | |
|---|---|
| STUDY TYPE: | **Subacute Inhalation – Rat** (82-4) |
| TOX. CHEM. NO.: | 497E |
| PC NUMBER: | 129059 |
| MRID NO.: | 422730-01 |
| TEST MATERIAL: | **NTN 33893 Technical** |
| SYNONYMS: | 1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine<br>Imidachloprid (proposed) |
| STUDY NUMBER: | T 3027635 |
| SPONSOR | Miles Corporation |
| TESTING FACILITY | Bayer AG Dept. of Toxicology, Wuppertal 1, W. Germany |
| TITLE OF REPORT | NTN 33893 (Proposed Common Name: Imidacloprid) Subacute Inhalation Toxicity Study in the Rat According to OECD Guideline No. 412. |
| AUTHOR | Dr. J. Pauluhn |
| REPORT ISSUED | July 18, 1989 |

CONCLUSIONS:

Groups of 10 male and 10 female Wistar rats were exposed (nose only) to analytical concentrations of 0.006, 0.031 or 0.191 mg/L, for 6hr/d, 5 d/wk 4 weeks.

NOEL: 0.191 mg/L
LOEL: >0.191 mg/L

This study satisfies the guideline requirements for an inhalation study in the rat (82-4) on the Technical, and is acceptable for regulatory purposes.

## MATERIALS

1. **Test Compound:** NTN 33893 Technical grade; Description: Solid—beige powder; Batch No. 180-587; Purity: 95.2%; Stability: assured to 6/7/88 (3 months beyond completion of study) at room temperature by analytical methods.

2. **Test Animals:** Species & Strain: Wistar Rat, Bor: WISW (SPF-Cpb); Weight when tested: 160 - 200 g; Source: Winkelmann Experimental Animal Breeders, Borchen.

3. **Environment:** Animals were housed in groups of five in Type III Makrolon® cages. Temperature: 22 ± 2°. Relative Humidity: approx. 50%. Photoperiod: 12 hour light-dark cycle (6am - 6pm). Air exchange: 10/hr. Food: Altromin® 1324 Maintenance Diet for Rats and Mice, available *ad libitum*. Water: Municipal (in watering bottles), available *ad libitum*.

## METHODS

1. **Dust Generation**

Air containing the test substance as dust was generated with a Wright Dust Feeder and an RGB brush-type generator[1] or an Exactomat 4200. The dust feeder had a dispersion pressure of about 120 Kpa; the air feed rate was about 30 l/min., and the exhaust rate was about 5 l/min. The test substance was continuously introduced into the inhalation chamber by the RGB 1000 generator with an air feed rate of 10 l/min. and an exhaust rate of 4 l/min. The test atmosphere generation conditions ensured at least 30 air changes/hr. (See Table 2 for concentration and partical size,

2. **Exposure and Observations**

Groups of 10 male and 10 female rats were exposed (nose only) to nominal concentrations of 0.005,0.030, or 0.180 mg test substance/L of air for 6 hours/day, 5 days/wk for four weeks. Animals were observed for signs of toxicity or mortality daily during the exposure period. Individual body weights were recorded just prior to exposure, and weekly thereafter. Ophthalmic examinations, clinical chemistry and hematology tests, urinalyses, gross pathological examinations, and organ withdrawals for gravimetric and histopathological examinations took place upon terminal sacrifice (by cardiac puncture under diethyl ether anesthesia).

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[1]Only used on the first day of exposure due to a defect in the Wright dust generator.

## RESULTS

1. **Clinical Observations**

No animals died during the study. No significant treatment-related clinical signs were observed during the study.

2. **Body Weight Gain**

Males in the high-dose group (0.180 mg/L) exhibited statistically significant ($p \leq 0.01$ at Weeks 1, 2, 3; $p \leq 0.05$ at Week 4) reductions in body weight gain. However, these reductions were all less than 10% (-5.8% to -8.6%), and thus of minimal toxicological significance.

Females at the high-dose level were not affected. Animals in the lower-dose groups were likewise unaffected by treatment.

3. **Ophthalmological examination**

No treatment related effects were observed in the 5 male and 5 female per group tested.

4. **Blood**

The CHECKED (X) parameters were examined.

a. **Hematology**

| | | | |
|---|---|---|---|
| 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) |
| | Erythrocyte count (RBC) | X | Mean corpusc. volume (MCV) |
| | Platelet count | X | Reticulocyte count |
| | Blood clotting measurements | | |
| | (Thromboplastin time) | | |
| X | (Clotting time) | | |
| | (Prothrombin time) | | |

No dose-related effects on hematological parameters were observed.

b. **Clinical Chemistry**

| | | | |
|---|---|---|---|
| X | **Electrolytes:** | X | **Other:** |
| X | Calcium | X | Albumin |
| X | Chloride | X | Blood creatinine |
| | Magnesium | X | Blood urea nitrogen* |
| | Phosphorous | X | Cholesterol* |
| X | Potassium | | Globulins |
| X | Sodium | X | Glucose* |
| | **Enzymes** | X | Total bilirubin* |
| X | Alkaline phosphatase (ALK) | X | Total serum Protein (TP) |
| | Cholinesterase (ChE) | | Triglycerides |
| X | Creatinine phosphokinase | | Serum protein electrophoresis |
| | Lactic acid dehydrogenase (LAD) | | |
| X | Serum alanine aminotransferase (also SGPT) | | |
| X | Serum aspartate aminotransferase (also SGOT) | | |
| | Gamma glutamyl transferase (GGT) | | |
| | Glutamate dehydrogenase | | |

No toxicologically significant changes in clinical chemistry were observed in the many endpoints examined. Any statistically significant changes observed occurred in one sex only (i.e., females), and/or were generally within the range of normal variation.

5. **Liver Tissue**

As seen in Table 1, induction of mixed function oxidase was observed in females at the two highest dose levels, and in males at the highest dose level. Indications of fatty degeneration of the liver were not observed.

TABLE 1. LIVER TISSUE TESTS (from Table #8 on page 58 of Report)

| Analytical Concentration (mg/L) | Study Week | Trigl mcmol/g | D-Den Mu/g | N-Den mU/g | P450 nmol/g |
|---|---|---|---|---|---|
| | | **Males** | | | |
| Air | 4 | 6.20 | 10.1 | 130.4 | 41.7 |
| 0.005 | 4 | 6.15 | 9.4 | 115.5 | 44.9 |
| 0.031 | 4 | 6.78 | 9.9 | 120.2 | 44.9 |
| 0.191 | 4 | 6.34 | 18.5** | 197.2** | 55.8** |
| | | **Females** | | | |
| Air | 4 | 6.19 | 9.5 | 59.7 | 37.2 |
| 0.005 | 4 | 6.06 | 8.3 | 59.7 | 42.2 |
| 0.031 | 4 | 5.89 | 9.4 | 73.6* | 36.7 |
| 0.191 | 4 | 5.76 | 11.5* | 105.4** | 39.1 |

6. **Urinalysis**

The CHECKED (X) parameters were examined.

| X | | X | |
|---|---|---|---|
| | Appearance | X | Glucose |
| X | Volume | X | Ketones |
| X | Specific Gravity | X | Bilirubin |
| X | Ph | X | Blood |
| X | Sediment (microscopic) | | Nitrate |
| X | Protein | X | Urobilirubin |

Urinary pH was elevated 13.8% ($p \leq 0.01$) in high-dose females only. No other significant effects were observed.

6. **Gross Pathology**

The CHECKED (X) parameters were examined

| X | | X | | X | |
|---|---|---|---|---|---|
| **Digestive system** | | **Cardiovasc./Hemat.** | | **Neurologic** | |
| X | Tongue | X | Aorta | XX | Brain |
| X | Salivary glands | XX | Heart | X | Peripheral Nerve |
| X | Esophagus | X | Bone marrow | X | Spinal c. (3 levels) |
| X | Stomach | X | Lymph nodes | X | Pituitary |
| X | Duodenum | XX | Spleen | X | Eyes (optic nerve) |
| X | Jejunum | | Thymus | **Glandular** | |
| X | Ileum | **Urogenital** | | XX | Adrenal gland |
| X | Cecum | XX | Kidneys | X | Lacrimal gland |
| X | Colon | X | Urinary bladder | X | Mammary gland |
| X | Rectum | XX | Testes | | Parathyroids |
| XX | Liver | X | Epididymides | X | Thyroid gland |
| X | Gall bladder | X | Prostate | **Other** | |
| X | Pancreas | X | Seminal vesicle | X | Bone |
| **Respiratory** | | XX | Ovaries | X | Skeletal Muscle |
| X | Trachea | X | Uterus | X | Skin |
| XX | Lung | | | | All gross lesions and masses |
| | Nose | | | | |
| | Pharynx | | | | |
| X | Larynx | | | | |

None of the animals contained any observable gross lesions.

8. **Histopathology**

No treatment-related lesions were observed.

9. **Organ Weights**

No treatment-related effects on absolute organ weights were observed. There was a statistically significant decrease in relative heart weights in females ($p \leq 0.01$) in the low and mid-dose groups (-7.1% and -6.9%, resp.), and in high-dose males (-5.3%, $p \leq 0.05$) These changes are not considered to be toxicologically significant, because they are small (males and females), and because a dose-response relationship was not observed (females).

10. **Particle Size** (Table 2)

TABLE 2. MEAN AEROSOL PARTICLE SIZES AS MEASURED DURING EXPOSURE (from Table #1 on page 47 of Report)

| Concentration, mg/L | | Mass Median Aerodynamic Diameter | Geometric Standard Deviation | % Particles < 1.1μ |
|---|---|---|---|---|
| Target | Mean Analytical | | | |
| 5 | 5.5± 1.2 | 2.37±0.34 | 1.54±0.02 | 95±2.1 |
| 30 | 30.5± 3.5 | 4.77±0.57 | 1.96±0.14 | 53±7.2 |
| 180 | 0.191±0.01 2.2 | 5.70±0.48 | 1.89±0.05 | 43±4.9 |

Due to the nature of the test compound, it was not possible to reduce the MMAD.

## DISCUSSION

The data can be summarized as follows in Table 3.

TABLE 3. SUMMARY OF EFFECTS OBSERVED FOLLOWING NTN INHALATION TREATMENT, AND THE DOSE LEVELS AT WHICH THEY OCCURRED

| Endpoint | NOEL, mg/L | LOEL, mg/L |
|---|---|---|
| Clinical Signs | 0.191 | >0.191 |
| Body Weight Gain | 0.191 | >0.191 |
| Ophthalmological | 0.191 | >0.191 |
| Blood | 0.191 | >0.191 |
| Liver | 0.005 | 0.031 |
| Urinalysis | 0.031 | 0.191 |
| Gross Pathology | 0.191 | >0.191 |
| Organ Weights | 0.191 | >0.191 |
| Histopathology | 0.191 | >0.191 |

From the above table it is apparent that the liver is the most sensitive organ. The authors conclude that there is functional hepatic impairment (of parenchyma) and that this is why other parameters show changes, such as lengthening of blood coagulation time and elevated urine pH levels, both in high-dose females. Since these changes were not accompanied by overt toxicity in either males or females such as clinical signs, body weight changes or lesions, it is concluded here that the **NOEL for this study is 0.191 mg/L**, the highest dose level tested. This dose may, however, be very close to the threshold of toxicity.

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FINAL

**DATA EVALUATION RECORD**

**IMIDACLOPRID**

**Study Type: 24-Month Oral Oncogenicity Study in Mice**

**Prepared for:**

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

**Prepared by:**

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer Pia Lindström Date 4/1/93
Pia Lindström, DPH

Independent Reviewer William L. McLellan Date 4/1/93
William McLellan, Ph.D.

QA/QC Manager Sharon Segal Date 4/1/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-66
Clement Numbers: 187 and 188
Project Officer: Caroline Gordon

EPA Reviewer: Ann Clevenger, Ph.D. Signature: Ann Clevenger
Review Section I, Toxicology Branch I/HED Date: 4/1/93

EPA Section Head: Marion Copley, D.V.M. Signature: Marion Copley
Review Section IV, Toxicology Branch I/HED Date: 4/9/93

**DATA EVALUATION RECORD**

STUDY TYPE: Oncogenicity study in mice; Guideline Series 83-2

EPA IDENTIFICATION NUMBERS

PC CODE NUMBER: 129099

TOX CHEM NUMBER: 497E

MRID NUMBERS: 422563-35 and 422563-36

TEST MATERIAL: 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazole-2-amine

SYNONYMS: Imidacloprid; NTN 33893

SPONSOR: Mobay Corporation, Stilwell, KS

STUDY NUMBERS: T5025710 (Main Study); T4029986 (Supplementary MTD Study)

TESTING FACILITY: Bayer AG, Wuppertal, Germany

TITLES OF REPORTS: NTN 33893 (Proposed Common Name Imidacloprid) Carcinogenicity Study on B6C3F1 Mice (Administration in the Food for 24 Months); NTN 33893 (Proposed Common Name: Imidacloprid) Carcinogenicity Study in B6C3F1 Mice (Supplementary MTD Testing for Study T 5025710 with Administration in Diet Over a 24-Month Period)

AUTHOR: B. Watta-Gebert

REPORTS ISSUED: January 28, 1991 and October 24, 1991

CONCLUSIONS: Dose Levels: Administered in diet,

Main Study: 0, 100, 330, or 1000 ppm
(males: 0, 20, 66, or 208 mg/kg/day;
(females: 0, 30, 104, or 274 mg/kg/day)

MTD Study: 0 or 2000 ppm
(males: 0 or 414 mg/kg/day
(females: 0 or 424 mg/kg/day)

Strain: B6C3F1
NOEL: 1000 ppm
LOEL: 2000 ppm, based on decreased body weight, food consumption, and water intake in both sexes

Administration of Imidacloprid at the stated dosages was not associated with an increase in tumor incidence when the treated animals were compared to the controls.

Classification: Core Guideline Data

This study does satisfy the guideline requirement for an oncogenicity study (83-2) in mice.

Special Review Criteria (40 CFR 154.7) None

A. MATERIAL

Test Compound

| | |
|---|---|
| Purity: | 95.0% and 95.3% |
| Description: | White to brownish solid |
| Batch number: | 180587 |
| Date received: | Not reported |
| Contaminants: | None reported |

Vehicle: Peanut oil (DAB 8)

Test Animals

| | |
|---|---|
| Species: | Mouse |
| Strain: | B6C3F1 |
| Age: | Approximately 5 weeks at start of main study and 7-8 weeks at start of MTD study |
| Weight: | Males--18-25 g; Females--14-18 g at start of main study<br>Males--19-31 g; Females--18-24 g at start of MTD study |
| Source: | Charles River Wiga GmbH, Sulzfeld, Germany (main study)<br>Winkelmann Experimental Animal Breeders, Borchen, Germany (MTD study) |

B. STUDY DESIGN

Animal Assignment: Animals were acclimated to laboratory conditions for 6 days (9 days for the MTD study) and were assigned by sex to the following test groups using a computer-generated randomization list:

| Test Group | Dosage in diet (ppm) | Number of Animals | |
|---|---|---|---|
| | | Males | Females |
| Main Study | | | |
| Control | 0 | 60 | 60 |
| Low-dosage | 100 | 60 | 60 |
| Mid-dosage | 330 | 60 | 60 |
| High-dosage | 1000 | 60 | 60 |
| MTD Study[a] | | | |
| Control | 0 | 60 | 60 |
| High-dosage | 2000 | 60 | 60 |

[a]A supplemental 'Maximum-Tolerated-Dose' experiment was conducted. Toxic effects observed in the high dosage group of the main study were not considered to be severe enough to have reached an MTD.

Environmental Conditions: Animals were housed under conventional conditions during the treatment period. Temperature was controlled at 22°± 2°C and humidity was approximately 50%. A 12/12 hour light/dark cycle was maintained. Air exchanges were approximately 10 per hour.

Dosage Rationale: Dosage levels were reported to have been selected based on the results of a subchronic feeding study in which mice received 0, 120, 600 or 3000 ppm of the test material in the diet. Compound-related effects were seen at 600 and 3000 ppm and were manifested as decreased body weight (600 and 3000 ppm) and increased mortality and clinical signs (3000 ppm). The subchronic study was not available for review. Selection of dosages for the MTD study were based on results of the main study and the subchronic feeding study.

Diet Preparation: The test compound was mixed into the food (Altromin® 1321 meal, Altromin GmbH and Co. KG, Lage) using a mixing granulator (main study) or a pelletizing mixer (MTD study). The mixtures also contained 1% ground nut oil to minimize dust formation. The food mixtures were prepared during the week preceding administration and were adjusted for active ingredient. Analysis for stability and homogeneity of the test material in the diet were conducted prior to the start of the study. Concentration was determined 19 times throughout the study.

Results: Analyses for concentration, homogeneity, and stability confirmed values within ±20% of nominals. Detailed results are reported below.

- Concentration. In the main study, the range (% of nominals) of concentrations for diets at all dosage levels was 81%-111%; at 100, 330, and 1000 ppm, the mean concentration (± coefficient of variance) was 96%±8.2%, 97%±7.1%, and 102%±6.4%, respectively. In the MTD study, the range (% of nominals) of concentrations at 2000 ppm was 90%-109%; the mean concentration was 1980 ppm with the mean % of nominal 99%±4.8%.

- Homogeneity. In the main study, homogeneity was analyzed at 50 and 1000 ppm. The range (% of nominals) of concentrations for both dosage levels was 102%-105%; at 50 and 1000 ppm, the means (± coefficient of variance) were 101%±1.1% and 102%±3.0%, respectively. In the MTD study, homogeneity was analyzed at 150 and 2400 ppm and at 50 and 1000 ppm. The range (% of nominals) of concentrations for both dosage levels was 102%-113%. At 150 and 2400 ppm, the means (± coefficient of variance) were 102%±1.7% and 107%±4.9%, respectively. At 50 and 1000 ppm, the means (± coefficient of variance) were 101%±1.1% and 102%±3.0%, respectively.

- Stability. In the main study, stability was analyzed at 50 and 1000 ppm. The range (% of nominals) of concentrations for both dosage levels after 14 days storage was 84%-87%. In the MTD study, stability was analyzed at 50 and 1000 ppm (14 days storage) and 2400 ppm (12 days storage). The ranges (% of nominals) of concentrations at 50 and 1000 ppm was 84%-85%; at 2400 ppm, it was 92%.

Food and Water Consumption: Animals received food (Altromin® 1321 meal) and tap water ad libitum throughout the study. Food was routinely analyzed for contaminants and nutritional quality.

Statistics: The following procedures were utilized.

- Body weights, food consumption, clinical chemistry, hematology, organ weights--Mann-Whitney U-test and Wilcoxon's test

- Survival curves--BMDP-Routine 1L followed by Wilcoxon's test and Kruskal-Wallis test

- There was no indication in the report that histologic findings in the main study were statistically analyzed; in the MTD study, Fishers's Exact test was used.

Compliance

- Signed Statements of No Data Confidentiality Claims, dated May 8, 1991 and December 11, 1991 were provided.

- Signed Statements of Compliance with EPA and OECD GLPs, dated January 22, May 8, October 17, and December 11, 1991 were provided.

- Signed Statements of Quality Assurance, dated January 25, and October 22, 1991 were provided.

C. METHODS AND RESULTS

Observations

Animals were observed twice daily during the week and once daily on weekends and holidays for mortality, moribundity and clinical signs of toxicity. Detailed physical examinations were carried out once a week.

Results: No compound-related mortalities or clinical signs were observed in either study for any sex or dosage level. In the main study, percent survival at week 104 was 84%-94% among all males and 80%-86% among all females. Cumulative mortality and percent survival are summarized in Table 1. The most frequently occurring clinical signs were hair loss, rough coat, and poor general condition. No effect on incidence, localization, or time of appearance of palpable masses was noted. In the main study, palpable masses were noted in 1, 3, 0, and 0 male(s) and in 3, 0, 2, and 1 female(s) at 0, 100, 330, and 1000 ppm, respectively. In the MTD study, palpable masses were noted in 0 and 1 males and in 2 and 1 females at 0 and 2000 ppm, respectively.

During the MTD study, animals at 2000 ppm developed a hypersensitivity to the ether anesthesia that was given at the time of tattooing (week 6). After 2 animals had died as a result of the anesthesia, tattooing was discontinued. This same hypersensitivity was also observed during the blood withdrawal. No compound-related mortality was observed during the MTD study. However, there was a significant indirect increase in male mortality at 2000 ppm because of deaths resulting from tattooing, blood withdrawal, and animals getting caught in the feeder. When these animals were excluded from the analysis, no differences were observed between the groups.

Body Weight

Body weight data were recorded prior to the start of the study and weekly thereafter, throughout the study. Terminal body weight data were also recorded. Body weight gain data were not provided.

Results: Compound-related effects on body weight were observed at 2000 ppm. Body weight data are summarized in Table 2.

In the main study among males, body weight at 1000 ppm was significantly ($p \leq 0.05$ or $\leq 0.01$) below control throughout most of the study (91%-95% of control) and during the first half of the study at 330 ppm (93%-97% of control). This latter finding (at 330 ppm) is most likely a result of the fact that body weight in this group was lower than control from the start of the study. These weight changes (at both 1000 and 330 ppm) were not considered to be biologically relevant since they were so small (1 g) and slight variations were already evident on day 0. Among females,

sporadic body weight changes at all dosage levels were observed and were considered to be unrelated to treatment. Sporadic weight changes at 100 ppm in both sexes were considered to be normal variations.

In the MTD study at 2000 ppm, body weights were consistently significantly ($p \leq 0.01$) lower than control from week 1 in both males and females. They were decreased by 13% and 11% at week 13 for males and females, respectively. Body weights gradually decreased over time from approximately 95% of control during week 1 to 75%-79% of control at time of terminal sacrifice.

Food Consumption and Compound Intake

In the main study, food consumption data were recorded weekly for 10 animals/sex/group through week 23 and for 20 animals/sex/group through the remainder of the study. Food efficiency was determined but no data were presented. In the MTD study, food consumption data were recorded for 20/animals/sex/group from the start of the study through week 103.

Results: Compound-related effects in food consumption were observed at 2000 ppm. Food consumption data (g/animal/day) are summarized in Table 3.

In the main study, sporadic, but significant changes ($p \leq 0.05$ or $\leq 0.01$) were observed in both sexes throughout the study at all dosage levels and were not considered to be treatment related. Overall food consumption at 1000 ppm in males (6.2 g/animal/day) was 95% of controls (6.5 g/animal/day) and in females (7.4 g/animal/day) was 90% of control (8.2 g/animal/day). Mean compound intake was 20.0, 65.6, and 208.2 mg/kg/day for males and 30.3, 103.6, and 274.4 mg/kg/day for females at nominal dietary levels of 100, 330, and 1000 ppm, respectively.

In the MTD study among males, food consumption was significantly ($p \leq 0.01$ or $\leq 0.05$; 90%) lower than control on most days throughout the first half of the study. Among females, food consumption was significantly ($p \leq 0.01$) lower than control (65%) throughout the entire study. The text indicated that food efficiency was lower than control among females (24%) at 2000 ppm but no data were presented for this parameter. Mean compound intake was 413.5 mg/kg/day for males and 423.9 mg/kg/day for females at 2000 ppm.

Water Intake

Water intake per group was determined every four weeks through week 101 in both studies.

Results: Some differences in water intake (g/animal/day) were observed at 1000 and 2000 ppm. In the main study among males, no effects were noted at any dosage level. Among females, water intake was approximately 10% lower than control at 1000 ppm, but this is probably not a biologically relevant change (data not shown). In

the MTD study, water intake was 29% and 38% lower than control at 2000 ppm among males and females, respectively, which was believed to be compound related.

Hematology and Clinical Chemistry

Blood was collected from the retro-orbital plexus for analyses of hematology parameters and most clinical chemistry parameters. Samples were taken from one of the caudal veins in nonfasted and nonanesthetized animals for the determination of glucose concentration . Samples were collected from 10 animals/sex/group after 12 and 24 months. An additional blood sample from 10 animals/sex/group in the control and high-dosage groups was taken after 18 months for a differential blood count. The following checked (x) parameters were determined.

Hematology

| | | | |
|---|---|---|---|
| X | Hematocrit (HCT) | X | Differential blood count |
| X | Hemoglobin (HGB) | X | Mean corpuscular HGB (MCH) |
| X | Leukocyte count (WBC) | X | Mean corpuscular HGB concentration (MCHC) |
| X | Erythrocyte count (RBC) | | |
| | Nucleated erythrocyte count (RBC) | X | Segmented neutrophil count (N-SEG) |
| X | Platelet count | X | Mean corpuscular volume (MCV) |
| | Reticulocyte count (RETIC) | | Prothrombin time (PT) |
| X | Red cell morphology | X | Basophil count (BASO) |
| X | Lymphocyte count (LYMP) | X | Monocyte count (MONO) |
| X | Eosinophil count (EOSN) | X | Band leucocyte (BAND) |

Results: No compound-related effects were observed in any hematological endpoint in either study for any sex or dosage level (data not shown). The sporadic differences from control were all within the range of historical controls.

Clinical Chemistry

| Electrolytes | Other |
|---|---|
| Calcium | Albumin |
| Chloride | Albumin/globulin ratio |
| Magnesium | X Creatinine |
| Phosphorus | X Urea |
| Potassium | X Cholesterol |
| Sodium | Globulin |
| | X Glucose |
| | X Bilirubin |
| Enzymes | Serum protein electrophoresis |
| | Phospholipids |
| X Alkaline phosphatase (ALP) | X Total protein |
| Cholinesterase | Triglycerides |
| Creatine kinase | |
| Lactate dehydrogenase (LDH) | |
| X Alanine aminotransferase (ALT/SGPT) | |
| X Aspartate aminotransferase (AST/SGOT) | |
| Gammaglutamyl transferase (GGT) | |

Results: In the main study, no effects were observed in either sex at any dosage level for any clinical chemistry parameter. In the MTD study, effects were observed in selected clinical chemistry parameters and included the following: Alkaline phosphatase levels increased while cholesterol levels decreased at 2000 ppm in both sexes at weeks 54 and 104. Among males, these changes were significant ($p \leq 0.01$) at both weeks 54 and 104. Among females, these changes were significant at week 54 ($p \leq 0.01$, alkaline phosphatase; $p \leq 0.05$, cholesterol) and nonsignificant at week 104. In addition, in females, bilirubin was elevated significantly at week 54 ($p \leq 0.01$) and nonsignificantly at week 104. All these changes in both sexes were not considered to be toxicological important since no gross or microscopic changes were observed in the liver.

Urinalysis

Urinalysis was not carried out in the main study. It was conducted in the MTD study. However, the results were not presented.

Sacrifice and Pathology

All animals found dead or sacrificed were subjected to a gross examination. Animals selected for interim sacrifice after 12 months and surviving animals at term were anesthetized with ether and exsanguinated. The checked (X) tissues were collected and preserved in 10% buffered formaldehyde solution for histological examination. In addition, the double checked (XX) organs were weighed.

| Digestive System | Cardiovasc./Hemat. | Neurologic |
|---|---|---|
| X Tongue | X Aorta | XX Brain |
| X Salivary glands[a] | X Heart[a] | X Peripheral nerve (sciatic nerve)[a] |
| X Esophagus[a] | X Bone marrow[a] | X Spinal cord (3 levels) |
| X Stomach[a] | X Lymph nodes[a] | X Pituitary[a] |
| X Duodenum[a] | XX Spleen | X Eyes, eyelids |
| X Jejunum[a] | X Thymus | X Optic nerve |
| X Ileum[a] | | |
| X Cecum[a] | Urogential | Glandular |
| X Colon[a] | | |
| X Rectum | XX Kidneys[a] | XX Adrenals[a] |
| XX Liver[a] | X Urinary bladder[a] | X Mammary glands[a] |
| X Gall bladder[a] | XX Testes[a] | X Thyroids[a] |
| X Pancreas[a] | X Epididymides | X Parathyroids[a] |
| X Residual intestine | X Prostate gland | X Harder's gland |
| | X Seminal vesicles | X Extraorbital gland |
| Other | XX Ovaries, oviduct | X Cymbocephalic glands |
| | X Uterus, vagina | Submaxillary gland |
| X Thigh muscle[a] | X Ureter | |
| X Skin[a] | X Urethra | Respiratory |
| X Head | | |
| X All gross lesion | | XX Lungs[a] |
| X Bone (femur and sternum)[a] | | X Larynx |
| X Tattooed auricles | | X Trachea[a] |

[a]Recommended by Subdivision F (October 1982) Guidelines.

Results--Organ Weights. Compound-related effects in organ weights were observed at 2000 ppm. However, for lack of accompanying gross and/or histopathological findings, these weight changes may not be of toxicological importance. In the main study, no compound-related effects in organ weights were seen for any sex or dosage level. Sporadic, but significant weight changes (all weights were within 11% of controls) were noted in all dosage groups and were frequently an indirect effect of decreased body weight. No consistent pattern was noted with regard to sex or dosage level, and therefore, these changes were not considered to be compound related. In the MTD study at 2000 ppm, weight changes in brain, liver, and spleen were observed more frequently in both sexes and were more severe (up to 40%) than those noted in the main study (up to 11% only). Overall, the females appeared to be more affected than the males. Selected organ weight data for both interim and terminal sacrifices in both studies are presented in Tables 4 (males) and 5 (females). Detailed results of all findings are presented in the text below.

In the main study among males, the following significant weight changes were reported: decreased absolute kidney weight at 1000 ppm and absolute testes weight at 100 ppm and increased relative brain weight at 1000 ppm (interim kill); decreased absolute and relative kidney weight at 1000 ppm

(terminal kill). Among females, the following significant weight changes were reported: decreased absolute liver weight at 1000 ppm and absolute and relative spleen weight at 100 ppm (interim kill); decreased absolute brain, adrenals, and liver weight at 1000 ppm, absolute brain, adrenal, and kidney weights at 330 ppm, and absolute kidney weight at 100 ppm (terminal kill); and decreased relative adrenal and liver weights at 1000 ppm, relative adrenal weight at 330 ppm, and relative kidney weight at 100 ppm (terminal kill).

In the MTD study among males, the following significant weight changes were reported: decreased absolute adrenal, lung, liver, spleen, and kidney weights; increased relative brain, spleen, and testes weights; and decreased relative liver weight (interim kill); decreased absolute lung, liver, spleen, and kidney weights; increased relative brain, lung, and testes weights (terminal kill). Among females, the following significant weight changes were reported: decreased absolute adrenal, lung, liver, spleen, and kidney weights; increased relative brain weight; and decreased relative liver and spleen weight (interim kill); decreased absolute brain, adrenal, lung, liver, spleen, kidney, and ovary weights; increased relative brain weight; and decreased relative liver and spleen weights (terminal kill).

Results--Gross Pathology. No compound-related gross findings were observed in either study for any sex or dosage level (data not shown).

In the main study at termination (including animals that died or were sacrificed moribund), the most frequent findings, observed in all dosage groups, included liver nodes and collapsed lungs in males and ovarian cysts in females. At interim sacrifice, ovarian nodes and/or cysts were noted in several females.

In the MTD study at termination (including animals that died or were sacrificed moribund), the most frequent findings, observed in all dosage groups, included liver and lung masses and lung discoloration in males and lung discoloration and ovarian cysts in females. At interim sacrifice, lung discoloration was noted in one male.

Results--Histopathology. No compound-related nonneoplastic or neoplastic findings were observed in either study for any sex or dosage level. Lesions were typical for mice at this age (data not shown). Tumor frequency and time-to-tumor appearance, as well as type and localization were similar in all groups. Some sex-related differences were noted. Total numbers of primary, benign, and malignant tumors as well as number of animals with these tumors, were similar across dosage groups.

- Nonneoplastic Lesions: In the main study at termination (including animals that died or were sacrificed moribund), the most frequent findings, observed in all dosage groups, included neuropathy, myelofibrosis, hemopoiesis of the spleen, tubular vacuolation and nephropathy of the kidney, tubular hyperplasia and cysts of the ovary, cystic hyperplasia of the uterus, and mineralization of the brain. Some of these findings were noted in only one sex. In

addition, leucocyte foci were observed in most organs. At interim sacrifice, similar findings were noted to a lesser degree.

In the MTD study at termination (including animals that died or were sacrificed moribund), the most frequent findings, observed in all dosage groups, included thalamic mineralization, congestive histiocytosis, lymphoid hyperplasia, and syncytial macrophages in the mesenteric lymph nodes, and polyluminal dilatation of the uterus. At interim sacrifice, similar findings were noted to a lesser degree. The following findings were frequent in the controls but infrequent at termination in the 2000 ppm males: epithelial vacuolization of the renal tubules, tubular basophilia in the renal pelvis, and lympho-cytosis in the renal pelvis. Other findings with a decreased frequency in males receiving 2000 ppm compared to controls were submucosal lymphocytosis of the urinary bladder and congestive histiocytosis in the mesenteric lymph nodes.

- Neoplastic Lesions: In the main study at termination (including animals that died or were sacrificed moribund), the most frequent tumors included pituitary adenoma (females); liver adenoma and carcinoma (males); adenoma in the harderian gland (both sexes); lung adenoma (males) and lymphomas of the hemoreticular system (mostly in females). At interim sacrifice, liver and lung adenomas and ovary teratoma were noted in several animals.

  In the MTD study at termination (including animals that died or were sacrificed moribund), the most frequent findings, observed in all dosage groups, included liver and lung adenomas and carcinomas (males mostly); pituitary adenoma (females); and malignant lymphoma in the hemopoietic system. At interim sacrifice, thyroid follicular cell adenoma was noted in one animal.

D. STUDY AUTHOR'S CONCLUSIONS

In the main study, Imidacloprid was administered to 50 male and 50 female mice in the diet at concentrations of 0, 100, 330, or 1000 ppm for 24 months. An additional 10 males and 10 females per dosage group were included for an interim sacrifice after 12 months. Compound-related effects, observed at 1000 ppm, were manifested as significantly decreased body weight/weight gain in males (10%) and females (5%) and slightly decreased food and water consumption in females. No compound-related effects were observed in mortality, clinical signs, clinical chemistry, hematology, organ weights, and gross and histopathological findings. The test material was not carcinogenic at any dosage level.

In an additional study, conducted to achieve an MTD, 50 male and 50 female mice were administered Imidacloprid in the diet at concentrations of 0 or 2000 ppm for 24 months. An additional 10 males and 10 females per dosage group were included for an interim sacrifice after 12 months. Compound-related effects, observed at 2000 ppm, were manifested as an increased incidence of clinical signs ("squeaking or twittering");

significantly decreased body weight/weight gain (26%-29%) and food consumption; and decreased water intake. No compound-related effects were observed in mortality, clinical signs, clinical chemistry parameters, hematology, organ weights, and gross and histopathological findings. The test material was not carcinogenic at any dosage level including 2000 ppm.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

The data reporting was thorough and the summary means that were validated were supported by the individual animal data. The chemical was tested at adequate dosage levels as evidenced by the decreased body weight. The reviewers agree with the study author's conclusions with one exception; organ weight changes in the liver, brain, and spleen at 2000 ppm were considered to be compound related but may have been secondary to effects on growth and general health condition. However, for lack of histopathological findings in these organs, the weight changes may not be toxicologically important.

The observed nonneoplastic lesions were within the commonly experienced range for this strain of mouse and were of no biological relevance. Incidences of tumors were similar across sexes and dosage groups for both studies.

The study is classified as Core Guideline. The NOEL and LOEL for systemic toxicity were 1000 and 2000 ppm, respectively, based on decreased body weight, food consumption, and water intake in both sexes.

TABLE 1. Cumulative Mortality and Percent Survival in Mice Fed Imidacloprid for 24 Months[a,b]

| Dietary Level (ppm) | Cumulative Mortality (Percent Survival) at Week: 26 | 52 | 78 | 104 |
|---|---|---|---|---|
| | | **Males** | | |
| Main Study | | | | |
| 0 | 0 (100) | 0 (100) | 0 (100) | 3 (94) |
| 100 | 0 (100) | 0 (100) | 1 (98) | 6 (88) |
| 330 | 0 (100) | 1 (98) | 1 (98) | 3 (94) |
| 1000 | 0 (100) | 0 (100) | 2 (96) | 8 (84) |
| MTD Study | | | | |
| 0 | 0 (100) | 0 (100) | 1 (98) | 6 (88) |
| 2000 | 4 (93) | 7 (88) | 9 (82) | 17* (66) |
| | | **Females** | | |
| Main Study | | | | |
| 0 | 1 (98) | 2 (97) | 2 (96) | 7 (86) |
| 100 | 1 (98) | 2 (97) | 2 (96) | 9 (82) |
| 330 | 0 (100) | 0 (100) | 2 (96) | 10 (80) |
| 1000 | 0 (100) | 0 (100) | 1 (98) | 9 (82) |
| MTD Study | | | | |
| 0 | 4 (93) | 4 (93) | 7 (86) | 19 (62) |
| 2000 | 4 (93) | 7 (88) | 8 (84) | 14 (72) |

[a]Data were extracted from Study No. T5025710, Table 2 and Study No. T4029986, pp. 106-113.

[b]N=60 weeks 0-52; N=50 weeks 53-104

*Significantly different from control ($p<0.05$)

TABLE 2. Mean Body Weight (kg ± S.D.) at Representative Intervals in Mice Fed Imidacloprid for 24 Months[a]

| Mean Body Weight Study Week: | Dose Level (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 100 | 330 | 1000 | 0[b] | 2000[b] |
| | | | **Males** | | | |
| 0 | 21 ± 1.0 | 20 ± 1.4 | 20 ± 1.4** | 20 ± 1.1 | 24 ± 2.7 | 25 ± 2.1** |
| 1 | 23 ± 0.9 | 24 ± 1.0 | 23 ± 1.0 | 23 ± 1.1 | 26 ± 2.0 | 24 ± 1.6** |
| 13 | 28 ± 1.3 | 28 ± 1.3 | 27 ± 1.2** | 27 ± 1.3** | 31 ± 2.1 | 27 ± 1.2** |
| 26 | 30 ± 2.0 | 30 ± 1.9 | 29 ± 1.6** | 29 ± 1.8** | 34 ± 3.3 | 28 ± 1.1** |
| 52 | 34 ± 3.2 | 32 ± 2.9 | 32 ± 3.3* | 31 ± 2.7** | 39 ± 5.2 | 29 ± 1.3** |
| 78 | 34 ± 3.3 | 34 ± 2.9 | 33 ± 3.5 | 33 ± 3.0* | 41 ± 6.0 | 31 ± 1.3** |
| 104 | 33 ± 3.5 | 33 ± 3.2 | 33 ± 4.0 | 33 ± 2.9 | 40 ± 6.3 | 30 ± 1.4** |
| | | | **Females** | | | |
| 0 | 16 ± 0.9 | 16 ± 0.8** | 16 ± 0.7** | 16 ± 0.6 | 21 ± 1.2 | 21 ± 1.2 |
| 1 | 19 ± 0.7 | 18 ± 0.8** | 18 ± 0.8** | 18 ± 0.8** | 22 ± 1.2 | 21 ± 1.5** |
| 13 | 24 ± 0.9 | 24 ± 0.9 | 24 ± 0.9** | 25 ± 0.9 | 27 ± 1.3 | 24 ± 1.1** |
| 26 | 26 ± 1.3 | 26 ± 1.2 | 26 ± 1.1** | 26 ± 1.0 | 28 ± 1.6 | 25 ± 1.3** |
| 52 | 29 ± 2.1 | 29 ± 2.4 | 28 ± 2.0** | 28 ± 1.7 | 32 ± 4.5 | 26 ± 1.0** |
| 78 | 29 ± 2.4 | 29 ± 2.6 | 29 ± 2.2 | 28 ± 2.3 | 34 ± 6.8 | 27 ± 1.1** |
| 104 | 29 ± 2.6 | 29 ± 1.5 | 29 ± 2.0 | 29 ± 2.3 | 34 ± 4.1 | 27 ± 1.2** |

[a]Data were extracted from Study No. T5025710, pp. 122-137, and Study No. T4029986, pp. 118-125.

[b]Represents dosage groups from the MTD study

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 3. Mean Food Consumption (g/animal/day ± S.D.) at Representative Intervals in Mice Fed Imidacloprid for 24 Months[a]

| Mean Food Consumption Study Week | Dose Level (ppm) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 100 | 330 | 1000 | 0[b] | 2000[b] |
| | | | **Males** | | | |
| 1 | 6.6 ± 0.7 | 6.4 ± 0.6 | 6.4 ± 0.7 | 6.1 ± 1.0 | 7.0 ± 1.4 | 4.4 ± 0.5** |
| 13 | 10.1 ± 2.9 | 11.9 ± 3.4 | 10.1 ± 3.1 | -- | 7.3 ± 1.6 | 5.9 ± 1.0** |
| 26 | 7.0 ± 1.1 | 6.5 ± 0.9 | 6.4 ± 1.3* | 6.4 ± 0.8 | 6.2 ± 0.9 | 5.8 ± 1.0 |
| 52 | 5.8 ± 0.7 | 5.4 ± 1.0 | 5.8 ± 1.1 | 5.5 ± 0.6 | 7.2 ± 0.8 | 6.5 ± 1.5* |
| 78 | 6.2 ± 0.8 | 6.1 ± 0.9 | 6.0 ± 0.5 | 6.2 ± 0.8 | 6.8 ± 1.3 | 5.8 ± 0.8* |
| 104[c] | 5.7 ± 1.3 | 5.5 ± 0.8 | 5.5 ± 1.2 | 5.0 ± 1.2 | 7.8 ± 1.0 | 7.5 ± 1.6 |
| | | | **Females** | | | |
| 1 | 8.0 ± 2.1 | 6.9 ± 1.0 | 7.6 ± 1.2 | 7.0 ± 1.5 | 7.8 ± 1.6 | 4.9 ± 0.8** |
| 13 | 11.7 ± 2.5 | 13.5 ± 1.3 | 10.8 ± 2.1 | 10.9 ± 2.5 | 9.6 ± 1.7 | 5.1 ± 0.7** |
| 26 | 8.7 ± 1.4 | 8.7 ± 1.4 | 9.1 ± 1.6 | 8.3 ± 1.3 | 8.5 ± 1.6 | 5.1 ± 0.8** |
| 52 | 7.7 ± 1.4 | 7.8 ± 1.7 | 7.6 ± 1.3 | 6.7 ± 1.6* | 8.8 ± 2.2 | 5.5 ± 0.7** |
| 78 | 8.0 ± 1.5 | 8.2 ± 1.5 | 8.4 ± 1.3 | 7.2 ± 1.4 | 7.8 ± 1.4 | 5.4 ± 0.8** |
| 104[c] | 7.0 ± 1.5 | 7.0 ± 1.4 | 7.5 ± 1.9 | 5.7 ± 0.9** | 9.9 ± 1.7 | 6.5 ± 1.6** |

[a]Data were extracted from Study No. T5025710, pp. 138-154, and Study No. T4029986, pp. 126-133.

[b]Represents dosage groups from the MTD study

[c]Food consumption values for the MTD study were from week 103.

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 4. Selected Organ Weights in Male Mice Fed Imidacloprid for 12 or 24 Months[a]

| Organ | Dose Level (ppm) 0 | 100 | 330 | 1000 | 0[b] | 2000[b] |
|---|---|---|---|---|---|---|
| | | | Interim Sacrifice (12 Months) | | | |
| Brain | | | | | | |
| Absolute (mg) | 491 ± 30 | 504 ± 19 | 500 ± 23 | 510 ± 17 | 489 ± 23 | 470 ± 16 |
| Relative (mg/100 g bw) | 1501 ± 196 | 1563 ± 182 | 1522 ± 188 | 1675 ± 97 | 1271 ± 156 | 1593 ± 62** |
| Liver | | | | | | |
| Absolute | 1433 ± 87 | 1422 ± 111 | 1454 ± 88 | 1371 ± 92 | 1811 ± 307 | 1265 ± 107** |
| Relative | 4356 ± 224 | 4395 ± 423 | 4403 ± 320 | 4499 ± 245 | 4634 ± 285 | 4283 ± 229* |
| | | | Terminal Sacrifice (24 Months) | | | |
| Brain | | | | | | |
| Absolute | 504 ± 23 | 501 ± 20 | 499 ± 23 | 503 ± 26 | 490 ± 26 | 483 ± 23 |
| Relative | 1522 ± 186 | 1516 ± 153 | 1482 ± 160 | 1528 ± 142 | 1250 ± 214 | 1631 ± 85** |
| Liver | | | | | | |
| Absolute | 1818 ± 767 | 1892 ± 924 | 1806 ± 813 | 1672 ± 281 | 1847 ± 204 | 1392 ± 114** |
| Relative | 5441 ± 2337 | 5721 ± 2812 | 5405 ± 2698 | 5067 ± 902 | 4553 ± 321 | 4687 ± 257 |

[a]Data were extracted from Study No. T5025710, pp. 180-187, and Study No. T4029986, pp. 151-158.

[b]Represents dosage groups from the MTD study

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

TABLE 5. Selected Organ Weights in Female Mice Fed Imidacloprid for 12 or 24 Months[a]

| Organ | Dose Level (ppm) 0 | 100 | 330 | 1000 | 0[b] | 2000[b] |
|---|---|---|---|---|---|---|
| | | | Interim Sacrifice (12 Months) | | | |
| Brain | | | | | | |
| Absolute (mg) | 510 ± 22 | 521 ± 15 | 512 ± 9 | 497 ± 23 | 499 ± 28 | 479 ± 22 |
| Relative (mg/100 g bw) | 1758 ± 163 | 1778 ± 112 | 1859 ± 89 | 1814 ± 55 | 1591 ± 110 | 1772 ± 66** |
| Liver | | | | | | |
| Absolute | 1369 ± 102 | 1349 ± 106 | 1337 ± 143 | 1271 ± 43* | 1447 ± 110 | 1126 ± 55** |
| Relative | 4709 ± 429 | 4582 ± 180 | 4839 ± 420 | 4641 ± 149 | 4600 ± 189 | 4163 ± 202** |
| Spleen | | | | | | |
| Absolute | 98 ± 10 | 87 ± 9* | 93 ± 16 | 88 ± 14 | 103 ± 10 | 76 ± 8** |
| Relative | 335 ± 39 | 298 ± 26* | 335 ± 58 | 319 ± 43 | 330 ± 37 | 282 ± 29* |
| | | | Terminal Sacrifice (24 Months) | | | |
| Brain | | | | | | |
| Absolute | 515 ± 21 | 511 ± 20 | 502 ± 25* | 506 ± 24* | 519 ± 28 | 488 ± 25** |
| Relative | 1752 ± 135 | 1747 ± 115 | 1722 ± 137 | 1741 ± 157 | 1552 ± 184 | 1785 ± 107** |
| Liver | | | | | | |
| Absolute | 1428 ± 192 | 1420 ± 328 | 1505 ± 562 | 1381 ± 250* | 1640 ± 217 | 1211 ± 101** |
| Relative | 4843 ± 533 | 4827 ± 916 | 5128 ± 1862 | 4737 ± 870* | 4832 ± 436 | 4417 ± 379** |
| Spleen | | | | | | |
| Absolute | 146 ± 77 | 149 ± 63 | 162 ± 91 | 138 ± 71 | 165 ± 40 | 97 ± 27** |
| Relative | 497 ± 249 | 511 ± 217 | 552 ± 299 | 476 ± 250 | 488 ± 120 | 352 ± 89** |

[a]Data were extracted from Study No. T5025710, pp. 181-187, and Study No. T4029986, pp. 151-158.

[b]Represents dosage groups from the MTD study

*Significantly different from control ($p \leq 0.05$)

**Significantly different from control ($p \leq 0.01$)

Reviewed by: Myron S. Ottley, Ph.D. MSOttley 4/20/93
Section IV, Tox Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (H7509C) Marion Copley 4/20/93

019537

# DATA EVALUATION REPORT

| | |
|---|---|
| STUDY TYPE: | **Developmental Toxicity—Rat** (83-3) |
| TOX. CHEM. NO.: | 497E |
| PC NUMBER: | 003125-URB |
| MRID NO.: | 422563-38 |
| TEST MATERIAL: | **NTN 33893 Technical** |
| SYNONYMS: | Imidacloprid (proposed)<br>1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine |
| STUDY NUMBER: | 083496 |
| SPONSOR: | Miles Corp. |
| TESTING FACILITIES: | RCC, Research and Consulting Company AG, Switzerland<br>RCC, UMWELTCHEMIE AG, Switzerland |
| TITLE OF REPORT: | Embryotoxicity Study (Including Teratogenicity) with NTN 33893 Technical in the Rat |
| AUTHOR(S): | H. Becker |
| REPORT ISSUED: | January 8, 1992 |

## CONCLUSIONS

NTN 33893 Technical was administered by gavage to HSD(SD) rats at 0, 10, 30, and 100 mg/kg/d during Gestational Days 6 - 16.

**Maternal**
NOEL = <10 mg/kg/d
LOEL = 10 mg/kg/d: decreased body weight gain. At 100 mg/kg/d: Decreased food consumption.
**Developmental**
NOEL = 30 mg/kg/d
LOEL = 100 mg/kg/d: increased wavy ribs.

CLASSIFICATION: **Core Minimum**
This study satisfies the guideline (83-3) requirements for a developmental toxicity study in the rat, and is acceptable for regulatory purposes.

## A. MATERIALS

1. **Test Compound:** NTN 33893 Technical; Description: White-grey crystals; Batch No: PT. 17001/87; Purity: 94.2%; Stability in vehicle: at least two hr. Stability of Pure Substance: at least until 9/27/87 (42 days after last sacrifice).

2. **Test Animals:** Species & Strain: Rat, HSD(SD); Age: Minimum 11 weeks; Weight at day 0 post coitum: 184 - 240 gm; Source: KFM, Kleintierfarm Madoerin AG, Switzerland.

## B. STUDY DESIGN

1. **Animal Assignment** Four groups of 25 dams were exposed to NTN 33893 Technical orally by gavage from days six through 15 post coitum as follows:

| | |
|---|---|
| Group 1 | 0 mg/kg body weight/day (vehicle control) |
| Group 2 | 10 mg/kg body weight/day |
| Group 3 | 30 mg/kg body weight/day |
| Group 4 | 100 mg/kg body weight/day |

2. **Dose Selection** was reportedly based on the results of a range finding study (RCC Project 083507). data were not submitted, nor were details provided. Dose volume remained a constant 10 ml/kg, and concentrations were adjusted daily to correspond with body weight.

3. **Food and Water** Standard Kliba 343 rat/mouse maintenance diet was available ad libitum. Tap water in bottles was also available ad libitum.

4. **Housing/Environmental Conditions** Animals were housed individually in Makrolon type 3 cages with wire mesh tops and granulated softwood bedding. Temperature range was 22 ± 3°C; Humidity range was 40% - 70%. Light/dark cycle was 12hr/12hr, with low-volume music played for eight hr during the light period.

5. **Statistical Analyses** were reportedly used to analyze body weights, food consumption, reproduction, and skeletal examination data:

   Univariate one-way analysis of variance was used to assess the significance of intergroup differences.

If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-one t-test), based on a pooled variance estimate, was applied for the comparison between the treated groups and the control group.

The Steel-test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.

Fisher's Exact test for 2x2 tables was applied if the variables could be dichotomized without loss of information.

Individual values, means, standard deviations and t-statistics were rounded off before printing.

## C. METHODS AND RESULTS

1. **Mating** Females were caged with sexually mature males (1:1) until evidence of copulation was present (spermatozoa in vaginal smear or vaginal plug). The day of mating was considered to be Gestational Day 0 (gd 0).

   Twenty five pregnant females were assigned to each group.

2. **Clinical Observations and Mortality** Animals were checked at least twice daily for signs of toxicity, or mortality. Animals sacrificed or found dead during the study were examined macroscopically, with emphasis on the uterus and its contents.

   No animals died during the study. No clinical signs of toxicity were observed during the conduct the study. No significant findings were made at necropsy. None of the maternal or reproductive parameters of toxicity was affected.

3. **Food Consumption** was recorded on gd 0, 6, 11, 16, and 21.

   As Tables 1 shows, mean food consumption decreased 10% or more relative to controls in all dose groups primarily during the initial days of treatment (gd 6 - 11), when measured on a g/animal basis. However, when food consumption is measured as a function of the animals' mean body weight for the time period (Table 2), food consumption was over 10% different from controls only in the high dose group (100 mg/kg/d). Therefore, the low effect level for food consumption is considered to be 100 mg/kg/d.

Toxicology Review # 010537 9/3/93

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The material not included contains the following type of information:

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____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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| TABLE 2. MEAN FOOD CONSUMPTION* (G/KG/D) DURING GESTATIONAL DAYS 0 THROUGH 21, AND PERCENT (%) DIFFERENCE FROM CONTROLS. | | | | | |
|---|---|---|---|---|---|
| **Dose Level** | **gd 0 - 6** | **gd 6 - 11** | **gd 11 - 16** | **gd 6 - 16** | **gd 16 - 21** |
| 0 mg/kg | 91.7 | 84.1 | 89.7 | 80.6 | 75.8 |
| 10 mg/kg | 92.0 (0) | 78.3 (-6.9%) | 94.4 (+5.2%) | 77.4 (-4.0%) | 81.6 (+7.7%) |
| 30 mg/kg | 91.0 (0) | 79.5 (-5.5%) | 88.1 (-1.8%) | 75.5 (-6.3%) | 80.9 (+6.7%) |
| 100 mg/kg | 92.2 (+1.0%) | 58.4 (-30.6%) | 76.8 (-14.4%) | 59.9 (-25.7%) | 96.5 (+27.3%) |

* Calculated by Reviewer; Statistics not evaluated.

4. **Body Weights** were recorded daily from gd 0 through 21.

Mean body weights were significantly ($p \leq 0.05$ or $p \leq 0.01$) less than controls only in the high-dose group from gd 8 through gd 20. However, actual losses were small, ranging from 4.3% on gd 8 to 7.3% on gd 17, and returning to 4.4% on gd 20.

Table 3 shows that mean body weight gain in the high-dose group was substantially (10% or more) below controls during gd 6 - 11 (-62.0%), gd 11 - 16 (-23.1%), and gd 6 - 16 (-42.4%).

In the mid-dose group (Table 3), mean body weight gain was substantially less than controls on gd 6 - 11 (-25.0%) and gd 6 - 16 (-11.2%). However, there was no diffence in mean body weight gain from gd 11 - 16.

In the low-dose group (Table 3), body weight gain was substantially less than controls during gd 6-11 (-21.7%) only.

Mean body weight gain (Table 3), when corrected (by subtracting uterine weight), was decreased only in the mid-dose (-28.7%) and high-dose (-47.0%) groups. The high-dose decrease was statistically significant ($p \leq 0.01$).

5. **Terminal Sacrifice and Postmortem Examination** Animals were sacrificed by $CO_2$ asphyxiation on gd 21 and fetuses were removed by Caesarean section. Female internal organs were examined macroscopically. Uterine contents, position of fetuses in the uterus and number of corpora lutea were noted and recorded.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

__/__ Information about a pending registration action.

__✓__ FIFRA registration data.

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Uteri with no apparent implantation sites were stained in aqueous ammonium sulfide solution to detect possible implantation sites. Offspring were removed from the uterus, weighed individually and observed for external malformations. Offspring were then prepared for soft tissue and skeletal evaluation according to standard teratological procedures.

a. **Female Reproduction Data** No significant difference from controls were observed in any of the treatment groups. The exception to the previous statement was the change ($p \leq 0.05$) in male:female ratio of pups: 58.6:41.4 in the high dose group, compared with 51.0:49.0 in controls. This observation is not considered to be treatment related, because in a preceding range-finding study, the male:female ratio was reported to be 50:50 at a dose level 50% higher (150 mg/kg/d) (Table 4).

b. **Fetal Data** No significant differences from controls were observed in fetal body weight Table 4), or during external and visceral (by Wilson technique) examinations (Table 5).

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_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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Skeletal examination revealed an increased incidence of wavy ribs (7 fetuses in 4 litters) compared with controls (2 fetuses in 1 litter) at the high-dose group (Table 6).

No other significant skeletal abnormalities were observed during the study.

TABLE 6. INCIDENCE OF WAVY RIBS IN RAT FETUSES OBSERVED FOLLOWING TREATMENT WITH NTN 33893 TECHNICAL

| Dose Level, mg/kg/d | Incidence in Fetuses / Fetuses Examined | Incidence in Litters / Total Litters |
|---|---|---|
| 0 | 2/158 | 1/25 |
| 10 | 1/155 | 1/25 |
| 30 | 0/153 | 0/24 |
| 100 | 7/149 | 4/25 |

Statistics Not Evaluated.

## D. SUMMARY

Table 7 summarizes the levels at which significant adverse effects were observed in female rats and their offspring.

TABLE 7. SUMMARY OF ADVERSE EFFECTS

| Endpoint Adversely Effected | LOEL, mg/kg/d | NOEL, mg/kg/day |
|---|---|---|
| Clinical Observations/Mortality | >100 | 100 |
| Food Consumption | 100 | 30 |
| Body Weight gains | 10 | >10 |
| Female Reproduction | >100 | 100 |
| Fetal Weight | >100 | 100 |
| Wavy Ribs | 100 | 30 |

NTN 33893 caused adverse effects in the rat conceptus only at dose levels that produced significant reductions in maternal food consumption and body weight gain. The author's conclusion is that NTN 33893 is not a developmental toxicant. It is not clear from the data provided however, whether the developmental effects observed were primary, or related to maternal toxicity.

# FINAL

DATA EVALUATION REPORT

IMIDACLOPRID

Study Type: Reproductive Toxicity

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

Principal Reviewer Pia Lindström Date 3/24/93
Pia Lindström, DPH

Independent Reviewer Sanjiv Diwan Date 3/24/93
Sanjiv Diwan, Ph.D.

QA/QC Manager Sharon A. Segal Date 3/25/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2- 66
Clement Number: 189
Project Officer: Caroline Gordon

Guideline Series 83-4: Reproductive Toxicity

EPA Reviewer: Myron Ottley, Ph.D.
Review Section IV, Toxicology Branch I/HED

Signature: MOttley
Date: 7/1/93

EPA Section Head: Marion Copley, D.V.M.
Review Section I, Toxicology Branch I/HED

Signature: Marion Copley
Date: 4/7/93

DATA EVALUATION REPORT

STUDY TYPE: Reproductive toxicity; Guideline Series 83-4

EPA IDENTIFICATION NUMBERS

PC Code: 129099

TOX CHEM. NUMBER.: 497E

MRID NUMBER.: 422563-40

TEST MATERIAL: 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine

SYNONYMS: Imidacloprid; NTN 33893

SPONSOR: Bayer Ag, Wuppertal, Germany

STUDY NUMBER: 100647

TESTING FACILITY: RCC, Research and Consulting Company AG, Itingen, Switzerland

TITLE OF REPORT: NTN 33893 Technical (Proposed C.N. Imidacloprid) Multiple Generation Reproduction Study in Rats

AUTHORS: P. Suter, K. Bierdermann, H. Luetkemeier, J.TH. Wilson, CH. Terrier

REPORT ISSUED: June 21, 1990

CONCLUSIONS: In a two-generation reproduction study, Wistar/Han rats were fed Imidacloprid in the diet at dosage levels of 0, 100, 250, or 700 ppm (during premating at 100, 250, and 700 ppm, for males ≈7.3, ≈18.3, and ≈52.0 mg/kg/day and for females ≈8.0, ≈20.5, and ≈57.4 mg/kg/day, respectively).

Parental NOEL = 700 ppm (≈55 mg/kg/day)
LOEL = Not determined

Reproductive NOEL = 100 ppm (≈ 8 mg/kg/day)
LOEL = 250 ppm (≈19 mg/kg/day), based on decreased pup body weight in both generations.

CLASSIFICATION: CORE Minimum Data. This study meets the minimum requirements set forth under Guideline Series 83-4 for a two-generation reproductive toxicity study in rats. The study has been classified as Minimum Data owing

to the following reporting deficiencies: No protocol was submitted and data supporting the stability of the test compound in the diet were not submitted (see page 8).

A. MATERIALS

Test Compound

| | |
|---|---|
| Purity: | 95.3% |
| Description: | Solid |
| Batch number: | Mischpartie 180587 |
| Date received: | Not reported |
| Contaminants: | None reported |

Vehicle: None used; the test material was administered in the diet.

Test Animals

| | |
|---|---|
| Species: | Rat |
| Strain: | Wistar/HAN |
| Source: | KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland |
| Age: | 4 weeks at delivery |
| Weight: | $F_0$ males--123-169 g at study initiation |
| | $F_0$ females--81-137 g at study initiation |

B. STUDY DESIGN

This study was designed to assess the potential of Imidacloprid to cause reproductive toxicity when administered continuously in the diet for two successive generations in rats.

Mating: After 10 days of acclimatization followed by 84 days of dietary treatment, $F_0$ females were mated with males from the same group in a ratio of 1:1 until a plug or sperm was detected in a vaginal smear (or for a maximum of 22 days). After delivery of the $F_{1A}$ pups, $F_0$ females were rested for two weeks and then mated again. During the second mating animals were paired with alternative partners. When possible, previously non-pregnant females and males failing to induce pregnancy were paired with previously successfully mated animals. Females, in which no evidence of mating was observed after 22 days, were paired a second time with alternative partners for a period of 22 days (maximum).

Following 105 days of dietary treatment, $F_1$ animals were paired one male to one female for a maximum of 21 days (sibling matings were avoided). Females in which no evidence of mating was observed were paired for a second time with alternative partners for a maximum of 4 days.

Environmental conditions: Temperature and humidity were maintained at 22°± 3°C and 40%-70%, respectively. There were 10-15 air changes per hour and a 12/12 hour light/dark cycle was maintained.

Group arrangement: $F_0$ animals were distributed using a random algorithm (computer-generated). $F_1$ animals were selected randomly according to RCC SOP. The groups were assigned as follows:

| Test Group | Dietary Level (ppm) | Number Assigned per Group $F_0$ Males | $F_0$ Females | $F_1$ Males | $F_1$ Females |
|---|---|---|---|---|---|
| Control | 0 | 30 | 30 | 26 | 26 |
| Low dose | 100 | 30 | 30 | 26 | 26 |
| Mid dose | 250 | 30 | 30 | 26 | 26 |
| High dose | 700 | 30 | 30 | 26 | 26 |

Dosage administered: The test material was administered in the diet (Kliba 343 rat/mouse maintenance diet, Klingentalmuehle Ag) for two consecutive generations. Diets were prepared at least every 2 weeks and stored at room temperature. The test material was mixed with granulated food in a Buehler mixer and pelleted in a Buehler pelleting machine. Water (1:10 volume/weight ratio) was used to achieve proper pelleting. Pellets were dried using warm air for 48-96 hours before storage. Analysis for concentration and homogeneity was performed prior to the start of the study, at the start of the prepairing and mating periods, and at the end of the gestation periods. Analysis for stability had been conducted in a previous study (RCC Project 087052).

Dosage rationale: Dosages were selected based upon a range-finding study (RCC Project 087052). The results of this study were not presented.

Observations: Observations for mortality, moribundity, and clinical signs of toxicity were conducted at least twice daily. Body weight data were recorded weekly for both males and females during premating but were not recorded during the mating periods. $F_0$ and $F_1$ females were weighed weekly during gestation. $F_0$ females were weighed on days 0, 4, 7, 14 and 21 postpartum and $F_1$ females were weighed on days 1, 4, 7, 14 and 21 postpartum. Male body weight data were recorded weekly for the remainder of the study. Food consumption data were recorded weekly with the exception of the mating periods. During the lactation period, food consumption data were only recorded until day 14 postpartum.

The following data were recorded for each litter:

- Number of live and dead pups, sex, and pup weight at birth and on lactation days 1, 4, 7, 14, and 21

- Gross and behavioral abnormalities

Uteri of apparently non-pregnant females were stained according to the method described by Salewski (1964) to detect early embryonic loss.

On day 4, pups were randomly culled to 4/sex/litter whenever possible. Culled pups and pups dying or killed during lactation were examined externally then sacrificed and examined for visceral abnormalities.

Twenty-six male and twenty-six female $F_1$ pups were randomly selected as $F_1$ parental animals. All $F_1$ pups not selected for the $F_1$ parental group or selected for histopathological examination were sacrificed and subjected to gross examination.

Parental animals of both generations and one pup/sex/generation/group were sacrificed and necropsied after weaning. The following tissues were preserved in 4% neutral phosphate buffered formaldehyde solution. Histopathology was carried out on these organs from the control and high-dose groups. Organs marked with an asterisk (*) were also weighed.

- Uterus
- Cervix
- Pituitary gland
- Prostate gland
- Gross lesions
- Thyroid gland
- *Liver
- *Ovaries
- Seminal vesicles w/coagulation gland
- *Testes w/epididymides
- Vagina

Statistical analysis: The following analyses were conducted.

- Body weight, food consumption, organ weights, clinical chemistry and hematology--ANOVA and Dunnett's test
- Reproductive parameters-- ANOVA based on Wilcoxon's ranks and Kruskall-Wallis' test
- Pup mortality--Fisher's exact test

Hematology and Clinical Chemistry: Blood samples were collected from 11 randomly selected animals/group/sex from the $F_1$ generation prior to necropsy. Blood samples were drawn from the retro-orbital plexus. Liver samples were also taken from these same animals to assay for triglycerides, cytochrome P-450, and N- and O-demethylase activity. The following parameters were determined:

Hematology

Erythrocyte count (RBC)
Hemoglobin (HB)
Hematocrit (HCT)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Nucleated erythrocytes normoblasts (NEN)
Total leukocyte count (WBC)
Differential leukocyte count
Red cell morphology
Thromboplastin time (PT)
Partial thromboplastin time (PTT)
Platelet count (PLATELETS)
Reticulocyte count (RETIC.)

Clinical Chemistry

Electrolytes

Calcium
Chloride
Potassium
Phosphorus
Sodium

Enzymes

Aspartate aminotransferase
Gamma glutamyl transferase
Alanine aminotransferase
Alkaline phosphatase
Creatinine kinase
Lactate dehydrogenase

Other

Glucose
Urea nitrogen
Creatinine
Globulin
A/G ratio
Albumin
Total protein
Total cholesterol
Total bilirubin
Triglycerides
Total lipids
Phospholipids

In Liver Tissue

Triglycerides
Cytochrome P-450
N-demethylase
O-demethylase

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated February 15, 1991, was provided.
- A signed Statement of Compliance with EPA, OECD, Japanese, and Swiss GLPs dated July 3, 1990 and February 15, 1991, was provided.
- A signed Quality Assurance Statement, dated January 22, 1990, was provided.

C. RESULTS

Test Material Analysis

Concentration and homogeneity analyses revealed concentrations from 81.4% to 111.1% of target. The study authors claim that the test material is stable for 21 days based on the results of a previous study (RCC Project 087052). However, results of this study were not presented.

Parental Toxicity

Mortality: No compound-related mortalities were observed in either sex or generation. Incidental deaths/moribund sacrifices are described below.

In the $F_0$ generation, one female from the control group was found dead on day 38 of the premating period. Necropsy revealed pelvic dilation and

kidneys, ureter, and bladder were filled with a yellowish turbid fluid. Another female from this same group was sacrificed moribund on day 21 of the $F_{1B}$ gestation period. Necropsy revealed collapsed lungs, reduced spleen size, and colon and ceceum distended with gas. A third female at 100 ppm was found dead on day 36 of the premating period. Necropsy revealed advanced autolysis and dilated bladder.

In the $F_1$ generation, one male at 100 ppm died following blood sampling. Necropsy did not reveal any abnormalities. One male at 250 ppm was sacrificed moribund on day 14 of the $F_{2B}$ postmating period. Necropsy revealed reduced spleen size and red-brown eschar and sores on the lips.

Clinical observations: No compound-related clinical signs were observed in either sex or generation. No summary data were provided. Hair loss and wounds were common findings in all groups (as stated in the text).

Body weight: Compound-related effects in body weight were observed at 700 ppm. Summaries of body weight and weight gain data for selected intervals are presented in Tables 1, 2, and 3. Detailed results are discussed below.

In the $F_0$ generation, among males at 700 ppm, body weight was significantly lower (5%-9%; Table 1) than control from day 8 of premating through day 56 of postmating, with the exception of days 22 and 29 postmating when the reduction in body weight was not significant (data not shown). Body weight gain was also 10% lower than control among males at 700 ppm on days 1-84 of the premating period.

Among $F_0$ females, body weight at 700 ppm was significantly lower than control on days 29, 36, 43, 57, 71, and 78 of the premating period (6%-7%; Table 1); days 0, 7, and 14 of the $F_{1A}$ gestation (7%; Table 2); days 0, 4, and 7 of the $F_{1A}$ lactation (5%-7%; Table 3); days 0, 7, 14, and 21 of the $F_{1B}$ gestation (5%-6%); and day 0 of the $F_{1B}$ lactation (5%). Weight gain at 700 ppm was 3%-12% lower than control during the premating and gestation periods and 19%-42% greater than control during the lactation periods.

In the $F_1$ generation at 700 ppm, male body weight was significantly lower than control on days 1, 8, 15, and 22 of the premating period (7%-8%; Table 1). Weight gain in all groups was comparable to control.

Among $F_1$ females, body weight was significantly lower (6%-9%) than control at 700 ppm during the entire premating period (Table 1) and $F_{2A}$ and $F_{2B}$ gestation periods (Table 2) and lactation periods (Table 3). Weight gain was 9%-12% lower than control during the premating and gestation periods and 38%-67% greater than control during the lactation periods.

Food consumption: No compound-related effects were observed in food efficiency (g/kg/day; data not shown). Decreased food consumption (g/animal/day) were noted at 700 ppm and followed a similar pattern as the decreased body weight discussed above (data not shown).

Compound intake: All values for mean compound intake were calculated by the reviewers using the summary group mean test article intake values.





60

In the $F_0$ generation, mean compound intake during premating was 8.1, 20.1, and 56.7 mg/kg/day for males and 8.8, 22.1, and 62.8 mg/kg/day for females at 100, 250, and 700 ppm, respectively. For females during $F_{1A}$ gestation mean compound intake was 7.7, 19.0, and 53.3 mg/kg/day and during $F_{1B}$ gestation it was 6.7, 17.0, and 46.0 mg/kg/day. During $F_{1A}$ lactation mean compound intake was 14.3, 38.3, and 101.3 mg/kg/day and during $F_{1B}$ lactation it was 14.0, 35.0, 95.7 mg/kg/day.

In the $F_1$ generation, mean compound intake during premating was 6.4, 16.5, and 47.3 mg/kg/day for males and 7.2, 18.9, and 52.3 mg/kg/day for females at 100, 250, and 700 ppm, respectively. For females during $F_{2A}$ gestation mean compound intake was 7.0, 18.3, and 50.3 mg/kg/day and during $F_{2B}$ gestation it was 6.7, 17.0, and 46.7 mg/kg/day. During $F_{2A}$ lactation mean compound intake was 15.0, 34.0, and 100.0 mg/kg/day and during $F_{2B}$ lactation it was 13.7, 33.3, and 98.3 mg/kg/day

Hematology: No compound-related effects were observed in any hematological parameter in $F_1$ males or females (data not shown). Incidental, but significant, findings consisted of the following:

| | | | | |
|---|---|---|---|---|
| Males | WBC | 700 ppm | ↑ | p≤0.05 |
| | EOSIN | 250 ppm | ↓ | p≤0.05 |
| | PT | 100 ppm | ↓ | p≤0.01 |
| Females | RETIC | 700 ppm | ↑ | p≤0.05 |
| | SEG | 700 ppm | ↓ | p≤0.05 |
| | LYMPH | 700 ppm | ↑ | p≤0.05 |
| | PTT | 100 ppm | ↑ | p≤0.05 |

Clinical Chemistry: No compound-related effects were observed in any clinical chemistry parameters in $F_1$ males or females (data not shown). Incidental, but significant findings, consisted of the following:

| | | | | |
|---|---|---|---|---|
| Males | Creatinine | 100 ppm | ↑ | p≤0.05 |
| | | 700 ppm | ↑ | p≤0.05 |
| | Chloride | 700 ppm | ↓ | p≤0.05 |
| | G-GLOB | 700 ppm | ↑ | p≤0.01 |
| | Cyt P-450 | 700 ppm | ↑ | p≤0.01 |
| | N-Demethyl | 700 ppm | ↑ | p≤0.05 |
| | O-Demethyl | 700 ppm | ↑ | p≤0.01 |
| Females | Glucose | 100 ppm | ↑ | p≤0.05 |
| | GPT | 250 ppm | ↓ | p≤0.01 |
| | CK | 100 ppm | ↓ | p≤0.05 |
| | | 250 ppm | ↓ | p≤0.01 |
| | | 700 ppm | ↓ | p≤0.05 |
| | ALP | 250 ppm | ↓ | p≤0.01 |
| | Potassium | 100 ppm | ↑ | p≤0.01 |
| | G-GLOB | 250 ppm | ↑ | p≤0.05 |
| | N-Demethyl | 250 ppm | ↓ | p≤0.01 |
| | O-Demethyl | 250 ppm | ↑ | p≤0.01 |
| | | 700 ppm | ↑ | p≤0.01 |

The increased cytochrome P-450 content in males and demethylase activity in both sexes at 700 ppm are indicative of increased metabolism in the

liver in response to metabolism of a xenobiotic. This was considered to be an adaptive response rather than a toxicological response.

Gross pathology: No compound-related gross findings were observed in either sex or generation.

Organ weights: No compound-related effects in organ weights were observed in either sex or generation. Relative testes weight was significantly ($p \leq 0.05$) lower than control at 100 ppm in the $F_1$ generation. Since this finding was not seen in the previous generation or in either of the two highest dosage groups, it is considered to be incidental. Absolute ovarian weight was significantly ($p \leq 0.01$) lower than control at 700 ppm in the $F_1$ generation. Since a similar reduction was not seen in the previous generation or in relative ovarian weight and because there were no related histopathology findings, this observation is not considered to be compound-related.

Histopathology: No compound-related histopathological findings were observed in either sex or generation. Frequent findings in both the control and high dosage groups of both generations included clear and mononuclear cells in the liver, testicular atrophy, and decreased sperm in the epididymides.

Reproductive Toxicity

Compound-related reproductive effects were observed at 250 and 700 ppm as significantly decreased body weights among all pups in all litters. At 100 ppm, weight reductions were also noted occasionally. But they were less consistent and therefore considered to be biologically irrelevant. Summaries of these effects are presented in Tables 4-7. Detailed results are presented in the text below.

In the $F_0$ generation among $F_{1A}$ pups (Table 4), mean pup body weight was significantly lower than control at 700 ppm on days 0-21 postpartum; at 250 ppm on day 0 postpartum; and at 100 ppm on day 21 postpartum. Among $F_{1B}$ pups (Table 5), mean pup body weight was significantly lower than control at 700 ppm on days 0, 7, 14, and 21 postpartum; at 250 and 100 ppm on days 0-7 postpartum.

In the $F_1$ generation among $F_{2A}$ pups (Table 6), mean pup body weight was significantly lower than control at 700 ppm on days 7-21 postpartum and at 250 ppm on day 7 postpartum. Among $F_{2B}$ pups (Table 7), mean pup body weight was significantly lower than control at 700 ppm on days 1-21 postpartum, at 250 ppm on day 21 postpartum; and at 100 ppm on day 1 postpartum.

No compound-related clinical signs, external anomalies, or behavior abnormalities were observed in any litter or generation.

Study/Reporting Deficiencies



A protocol was not submitted. Results of the analysis for stability of the test material in the diet were not presented. However, since this information is available from other studies on NTN 33893 technical (see MRID# 422563-31 and 422563-32, which have study #s 100562 and 101931,

respectively), this deficiency will not alter the Core grading of this study.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Test Material Analyses: Concentration and homogeneity of the test material in the diet were confirmed to be overall within ±20% of nominal values. Results of the stability analysis were not presented.

Parental Toxicity: No parental toxicity was observed in this study. Body weights and food consumption were significantly decreased at 700 ppm in both sexes and generations. Food efficiency was not affected. The decreased food consumption was most likely due to non-palatability of the test compound which resulted in decreased body weight in both sexes and generations. No compound-related effects were seen in mortality, clinical signs, hematology parameters, organ weights, or gross or microscopic observations. Increased activity of selected liver enzymes was observed in both sexes at 700 ppm. In the absence of increase in liver-derived plasma enzymes, plasma bilirubin, liver triglycerides, changes in liver morphology, and organ weight, this was considered to be a physiological adaptation to the test compound rather than a toxicological response. Based on these results, the NOEL for parental toxicity was 700 ppm; the LOEL was not determined.

Reproductive Toxicity: Compound-related reproductive toxicity was observed at 250 and 700 ppm. It was manifested as decreased pup body weight. No compound-related effects were observed for any other reproductive parameter. Based on these results, the NOEL and LOEL for reproductive toxicity were 100 and 250 ppm, respectively.

E. CLASSIFICATION: CORE Minimum Data.

Parental toxicity NOEL = 700 ppm
Parental toxicity LOEL = Not determined

Reproductive toxicity NOEL = 100 ppm
Reproductive toxicity LOEL = 250 ppm (based on decreased pup body weight)

F. RISK ASSESSMENT: Not applicable

Coded slides were scanned under oil immersion and morphologically normal (2N=22±1) metaphases scored for the conventional array of structural chromosome aberrations (primary cytogenetic damage), as well as for mitotic index (as a measure of cytotoxicity).

This lab considers a test substance positive (in an acceptable assay only) if it induces either a significant dose-related increase in the number of aberrations, or a significant and reproducible positive response for at least one of the test points. A test article producing neither a significant dose-related increase in the number of structural chromosomal aberrations nor a significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system. This is confirmed by means of the nonparametric Mann-Whitney test.

E. RESULTS: In preliminary dose selection tests, WAK 3839 precipitated at concentrations of 1,300 ug/ml, a toxic dose in the absence of activation (Report Tables 1 to 3). However, in contrast to the significant positive results in response to the referenced mutagens, at no dose in replicate experiments with/without activation, did the test article induce increased structural chromosomal aberrations above solvent control values (Report Tables 4 through 23, summarized in Tables 24, 25 and 26, attached here). The authors concluded that the test article was not clastogenic in V79 cells up to the limits of solibility in tissue culture medium.

F. TB EVALUATION ACCEPTABLE



ATTACHMENT: (Data Tables)

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Toxicology Review # 010537 9/3/93

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010537

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

E1-25-93

8/27/93

DATA EVALUATION RECORD

MRID NUMBER No.:422563-71
PC No.:129099
RD Record No.:S419490
EPA ID No.:003125-URU (NTN 33893 Tech)

Tox Chem. No.:497E
Project No.:D180229/D179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity --Chromosome aberrations in vitro (CHO cells)

CHEMICAL: NTN 37571 (metabolic of NTN 33893)

SYNONYMNS: WAK 3839

SPONSOR: Mobay, KC

TESTING FACILITY: Nihon Tohushu Noyaku Seizo KK (Japan)

TITLE OF REPORT: In Vitro Cytogenetic Assay Measuring Chromoscme Aberrations in CHO-K1 Cells

AUTHOR: M. Usami

STUDY NUMBER: 88P016 (Report #RP880088/100678)

DATE ISSUED: November 5, 1988

CONCLUSIONS: Negative for inducing chromosome aberrations in Chinese hamster ovary (CHO) cells exposed up to cytotoxic levels (1000 ug/ml), with or without mammalian metabolic activation.

TB-I EVALUATION: ACCEPTABLE

II. DETAILED REVIEW

A. TEST MATERIAL: NTN 37571 (Wako Chemical)

Description: White-yellow powder
Batches (Lots): WAK 3839
Purity (%): 98.8
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. TEST ORGANISM: Established mammalian cell strain

Species: Chinese hamster (ovary)
Strain: CHO-K1
Source: Dainippon Pharmaceutical

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the clastogenic potential of the test article when administered in vitro to Chinese hamster ovary (CHO) cells, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: After preliminary dose-selection testing, duplicate cultures of CHO cells were exposed to graded concentrations of test article in the absence of activation for 24 or 48 hours, but only for 4 hour (plus 20 hour post-treatment incubation in fresh tissue culture medium) in the presence of a mammalian metabolic activation system (rat liver S9, purchased from Kikkomen) plus NADP(H)-generating co-factors. The mammalian metabolic activation system (rat liver S9, purchased from Kikkomen) plus NADP(H)-generating co-factors. The mutagens, MNNG (methyl-nitro-nitrosoguanidine) and DMN (dimethylnitrosamine) served as positive controls for, respectively, the non-activation and activation series.

E. RESULTS: In the preliminary dose-selection tests, the test article was moderately toxic at 1000 ug/ml (relative growth rate = 50%) without activation but only minimally (84% RG) +S9 (Tables 1, 3). However, in contrast to the statistically increased frequency of aberrations produced by the positive control substances, no increased clastogenesis over control values was found with the test article. (Tables 2 and 4, attached here)

F. TB EVALUATION: Acceptable

ATTACHMENTS (Data Tables)

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Toxicology Review # 010537 9/3/93

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010537

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

01-12-93

DATA EVALUATION RECORD

MRID NUMBER No.: 422563-72
PC No.: 129099
RD Record No.: S419490
EPA ID No.: 003125-URU (NTN 33893 tech)
Tox Chem. No.: 475E
Project No.: D180229/D179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---DNA damage/repair in vitro (HPC/UDS)

CHEMICAL: WAK 3839 (metabolite of NTN 33893)

SYNONYMNS: NTN 37571

SPONSOR: Mobay, KC

TESTING FACILITY: Cytotest Cell Research, Darmstadt (FRG)

TITLE OF REPORT: Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro with WAK 3839.

AUTHOR: R. Fautz

STUDY NUMBER: T4030074 (Report # R4746/100665)

DATE ISSUED: April 24, 1989

CONCLUSIONS: Negative for induced DNA damage repair in rat hepatocyte cultures, as represented by increased grain counts indicating unscheduled DNA synthesis, treated up to cytotoxic doses (1333 ug/ml)

TB-I EVALUATION: ACCEPTABLE

## II. DETAILED REVIEW

A. TEST MATERIAL: WAK 3839 (Wako Chemical)

| | |
|---|---|
| Description: | White-yellowish powder |
| Batches (Lots): | WAK 3839/C-E |
| Purity (%): | 98.9 |
| Solvent/carrier/diluent: | Dimethylsulfoxide (DMSO) |

B. TEST ORGANISM: Primary hepatocyte cultures

| | |
|---|---|
| Species: | Rat |
| Strain: | Wistar CF HB |
| Age: | 8-12 weeks |
| Weights - males: | 180-240 g |
| Source: | SAVO Ivanovas (Kisslegg FRG) |

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the genotoxic potential of the test article when administered in vitro to cultures of primary rat hepatocytes, and measuring unscheduled DNA synthesis (as increased nuclear silver grain count), according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following cytotoxicity testing, hepatocytes isolated from male rats were exposed as coverslip cultures for 18 hours to graded concentrations of test article together with tritiated thymidine ($^3$H-TdR, 5uCi/ml; sp. act., 20 Ci/mmol). After this treatment, coverslip cultures were expanded in 1% sodium citrate, fixed in Carnoy's and mounted on standard glass microscope slides. The slides were then dipped (under safelight) in photographic emulsion (Ilford K-2), dried and stored under refrigeration in light-tight boxes. After seven days storage, slides were developed in standard photographic solutions, stained with aceto-orcein, and covered for microscopic examination.

Under oil immersion, at least 100 cells per dose level were scored for photographic silver grains over hepatocyte nuclei, and net nuclear grain counts (NNGC) determined (as a measure of unscheduled DNA synthetic repair of damage). The

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mutagen 2-acetoaminofluorene (AAF) served as positive control. The assay was repeated twice (three trials in toto).

E. RESULTS: In preliminary testing, doses of 444 mg/ml and above were toxic, and 1333 mg/ml and above precipitated out. Hence in the repeat experiments the following doses were scored:

| | |
|---|---|
| EXP-I: | 8 concentrations from 0.04 to 133.33 ug/ml |
| EXP II: | 10 concentrations from 0.04 thru 1333.33 ug/ml |
| EXP III: | 5 concentrations from 13.33 to 1333.33 ug/ml |

Although isolated increased grain counts were encountered in the first two trials, no reproducible dose-dependent increases in NNGC were found (see Data Tables, attached here). The reference mutagen, AAF, produced significantly increased UDS (as measured by grain counts).

F. TB EVALUATION: ACCEPTABLE.

ATTACHMENT (Data Tables

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100665

010537

# TABLES OF RESULTS

## EXPERIMENT I

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Reviewed by: Irving Mauer, Ph.D., Geneticist,
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C)

03-24-93
8/27/93

DATA EVALUATION REPORT

I. SUMMARY

Study Type: (84-2) Mutagenicity - Chromosome aberrations in vivo (Mouse MT)

MRID No.: 422563-66
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.:D180299/D179336

Chemical: WAK 3839 (metabolite of NTN 33893)

Synonymns: NTN 37571

Sponsor: MOBAY (Miles), Kansas City

Testing Facility: Bayer AG, Wuppertal (FRG)

Title of Report: WAK 3839 or NTN 37571: Micronucleus Test on the Mouse After Intraperitoneal Injection

Author: B. A. Herbold

Study No.: T0032852 (Report No. 18407/100664)

Report Issued: October 3, 1989

TB Conclusions: Negative for inducing micronuclei in PCE of mice treated parenterally at the MTD (50 mg/kg)

TB-I Evaluation: Acceptable

II. DETAILED REVIEW:

A. Test Material: WAK 3839 (Bayer AG)

Description: Yellow-greenish powder
Batch (Lot): WAK 3839/C-E
Purity (%): 98.9
Solvent/carrier/diluent: 0.5% Aqueous Cremophor Emulsion (CMC)

B. Test Organism: Rodent

Species: Mouse
Strain: Bor:NMRI (SPF Han)
Age: 8-12 weeks
Weights: Males/females: 31-41 g
Source: F. Winkelman, Borchen (FRG)

C. Study Design (Protocol):

This study was designed to assess the clastogenic potential of the test article when administered i.p. to mice, and evaluating the induction of micronuclei in PCE, according to established (published) procedures and FIFRA Test Guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following dose-selection testing, groups of mice (5 male: 5 female/group) were injected once i.p. with 50 mg/kg test article, and sacrificed 24, 48 or 72 hours later. Two further groups were given Cremophor or cyclophosphamide (CP) to serve as negative and positive controls, respectively.

At sacrifice, femoral bone marrow was prepared by standard cytological procedures as smears on microscope slides, stained with H and E, and sealed under coverslips. One thousand polychromatic erythrocytes (PCE) per animal on coded slides were scored for the presence of micronuclei (m-PCE), as well as determining the ratio of PCE to normochromatic erythrocytes (indirect evidence of cytotoxicity).

Wilcoxon's (non-parametric) Rank Sum Test was used to analyze the resulting data, with alpha set at 5%.

Conventionally acceptable criteria for assay acceptance as well as for responses were provided in the Final Report.

E. Results: In the preliminary pilot (dose-selection) study, all animals given 100 mg/kg i.p. died, but only 1 of 10 at 50 mg/kg. Dose-related clinical toxicity was noted, starting at 25 mg/kg, as follows: Apathy, staggering gait, rales. Hence 50 mg/kg was selected at the MTD for the main study.

The same syndrome of adverse clinical signs was evident in the main study at 50 mg/kg in all groups, but no mortalities. Whereas the ratio of PCE to NCE was slightly altered (indicating that the test article reached the target tissue to produce toxicity) no statistically significant or biologically relevant differences from negative control values were recorded in any test group (Report tables 1 through 5, attached here). By contrast, the positive control, CP, manifested clearly clastogenic effects.

Hence, the author concluded that WAK 3539 was not clastogenic in inducing micronuclei in bone marrow cells of mice treated parenterally at a clearly toxic dose.

F. TB-I Evaluation: Acceptable

Attachments (Data Tables)

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Table 1. Body Weight (g ± S.D.) During the Premating Period for Rats Fed Imidacloprid for Two Successive Generations[a]

| Study Days | Dietary Level (ppm) 0 | 100 | 250 | 700 |
|---|---|---|---|---|
| **$F_0$ Males** | | | | |
| 1 | 148 ± 9 | 146 ± 10 | 148 ± 11 | 147 ± 9 |
| 8 | 192 ± 12 | 188 ± 13 | 188 ± 15 | 182 ± 11* |
| 29 | 292 ± 21 | 289 ± 22 | 283 ± 20 | 269 ± 25* |
| 57 | 365 ± 28 | 367 ± 32 | 345 ± 43 | 335 ± 35* |
| 84 | 396 ± 33 | 401 ± 36 | 382 ± 31 | 369 ± 37* |
| Wt. Gain 1-84[b] | 248 | 255 | 234 | 222 |
| **$F_0$ Females** | | | | |
| 1 | 112 ± 8 | 117 ± 8* | 117 ± 6* | 114 ± 8 |
| 8 | 135 ± 11 | 140 ± 8 | 140 ± 8 | 134 ± 8 |
| 29 | 185 ± 19 | 188 ± 13 | 188 ± 14 | 173 ± 13* |
| 57 | 219 ± 26 | 221 ± 13 | 223 ± 20 | 206 ± 14* |
| 84 | 230 ± 30 | 232 ± 14 | 236 ± 24 | 218 ± 14 |
| Wt. Gain 1-84 | 118 | 115 | 119 | 104 |
| **$F_1$ Males** | | | | |
| 1 | 251 ± 23 | 257 ± 22 | 242 ± 18 | 230 ± 26* |
| 8 | 285 ± 25 | 293 ± 25 | 278 ± 21 | 265 ± 27* |
| 29 | 352 ± 32 | 363 ± 33 | 344 ± 31 | 330 ± 34 |
| 57 | 404 ± 38 | 419 ± 41 | 396 ± 38 | 379 ± 43 |
| 85 | 433 ± 40 | 450 ± 45 | 426 ± 44 | 412 ± 49 |
| 105 | 449 ± 42 | 468 ± 48 | 445 ± 46 | 427 ± 48 |
| Wt. Gain 1-105 | 198 | 211 | 203 | 197 |
| **$F_1$ Females** | | | | |
| 1 | 179 ± 14 | 176 ± 14 | 175 ± 14 | 165 ± 16* |
| 8 | 195 ± 15 | 192 ± 15 | 190 ± 15 | 178 ± 17* |
| 29 | 225 ± 17 | 223 ± 19 | 218 ± 17 | 204 ± 20* |
| 85 | 259 ± 20 | 256 ± 22 | 252 ± 18 | 235 ± 22 |
| 57 | 245 ± 19 | 243 ± 20 | 240 ± 17 | 226 ± 22* |
| 105 | 267 ± 21 | 263 ± 23 | 261 ± 19 | 244 ± 2?* |
| Wt. Gain 1-105 | 88 | 87 | 86 | 79 |

[a]Data were extracted from Study No. 100647, pp. 89, 90, 93, 94, 125, 133, and 134.

[b]Standard deviation for weight gain was not provided.

*Significantly different from control (p≤0.05)

Table 2. Body Weight (g ± S.D.) During Gestation for Rats Fed Imidacloprid for Two Successive Generations[a]

| Study Days | Dietary Level (ppm) 0 | 100 | 250 | 700 |
|---|---|---|---|---|
| $F_0$ Generation-$F_{1A}$ litters | | | | |
| 0 | 230 ± 37 | 230 ± 15 | 235 ± 22 | 214 ± 17* |
| 7 | 248 ± 36 | 246 ± 16 | 253 ± 24 | 231 ± 17* |
| 14 | 274 ± 37 | 273 ± 17 | 282 ± 28 | 255 ± 19* |
| 21 | 333 ± 43 | 332 ± 22 | 344 ± 32 | 314 ± 24 |
| Wt. Gain 0-21[b] | 103 | 102 | 109 | 100 |
| $F_0$ Generation-$F_{1B}$ litters | | | | |
| 0 | 254 ± 19 | 255 ± 13 | 257 ± 20 | 240 ± 18* |
| 7 | 268 ± 21 | 269 ± 14 | 273 ± 20 | 255 ± 19* |
| 14 | 294 ± 23 | 294 ± 15 | 298 ± 22 | 278 ± 21* |
| 21 | 363 ± 34 | 364 ± 19 | 367 ± 30 | 342 ± 25* |
| Wt. Gain 0-21 | 109 | 109 | 110 | 102 |
| $F_1$ Generation-$F_{2A}$ litters | | | | |
| 0 | 260 ± 21 | 257 ± 21 | 261 ± 14 | 234 ± 23* |
| 7 | 274 ± 22 | 268 ± 22 | 274 ± 16 | 246 ± 23* |
| 14 | 295 ± 24 | 288 ± 22 | 294 ± 18 | 265 ± 24* |
| 21 | 356 ± 31 | 350 ± 28 | 355 ± 26 | 321 ± 31* |
| Wt. Gain 0-21 | 96 | 93 | 94 | 87 |
| $F_1$ Generation-$F_{2B}$ litters | | | | |
| 0 | 279 ± 26 | 278 ± 28 | 272 ± 18 | 257 ± 22* |
| 7 | 297 ± 29 | 293 ± 26 | 287 ± 20 | 271 ± 24* |
| 14 | 322 ± 31 | 315 ± 27 | 309 ± 22 | 291 ± 27* |
| 21 | 384 ± 41 | 374 ± 31 | 362 ± 30 | 349 ± 35* |
| Wt. Gain 0-21 | 105 | 96 | 90 | 92 |

[a]Data were extracted from Study No. 100647, pp. 127, 129, 130, 132, 135, 137, and 140.

[b]Standard deviation for weight gain was not provided.

*Significantly different from control ($p \leq 0.05$)

Table 3. Body Weight (g ± S.D.) During Lactation for Rats Fed Imidacloprid for Two Successive Generations[a]

| Study Days | Dietary Level (ppm) 0 | 100 | 250 | 700 |
|---|---|---|---|---|
| **$F_0$ Generation-$F_{1a}$ litters** | | | | |
| 0 | 247 ± 30 | 249 ± 15 | 253 ± 26 | 230 ± 18* |
| 4 | 264 ± 29 | 262 ± 17 | 276 ± 25 | 245 ± 16* |
| 7 | 273 ± 26 | 274 ± 18 | 288 ± 25 | 258 ± 17* |
| 14 | 284 ± 30 | 285 ± 21 | 295 ± 22 | 270 ± 19 |
| 21 | 279 ± 30 | 278 ± 20 | 290 ± 23 | 268 ± 19 |
| Wt. Gain 0-21[b] | 32 | 29 | 37 | 38 |
| **$F_0$ Generation-$F_{1b}$ litters** | | | | |
| 0 | 274 ± 21 | 277 ± 21 | 281 ± 25 | 259 ± 19* |
| 4 | 292 ± 23 | 293 ± 19 | 301 ± 23 | 281 ± 21 |
| 7 | 298 ± 22 | 301 ± 22 | 309 ± 25 | 288 ± 20 |
| 14 | 310 ± 25 | 313 ± 20 | 319 ± 22 | 297 ± 18 |
| 21 | 298 ± 23 | 300 ± 19 | 310 ± 20 | 293 ± 21 |
| Wt. Gain 0-21 | 24 | 23 | 29 | 34 |
| **$F_1$ Generation-$F_{2a}$ litters** | | | | |
| 0 | 269 ± 26 | 258 ± 25 | 261 ± 19 | 239 ± 22* |
| 4 | 283 ± 25 | 276 ± 25 | 280 ± 19 | 256 ± 22* |
| 7 | 288 ± 25 | 283 ± 24 | 286 ± 19 | 263 ± 23* |
| 14 | 302 ± 24 | 296 ± 24 | 299 ± 16 | 277 ± 19* |
| 21 | 295 ± 22 | 292 ± 25 | 293 ± 18 | 275 ± 20* |
| Wt. Gain 0-21 | 26 | 34 | 32 | 36 |
| **$F_1$ Generation-$F_{2b}$ litters** | | | | |
| 0 | 294 ± 29 | 289 ± 28 | 284 ± 22 | 262 ± 23* |
| 4 | 312 ± 29 | 308 ± 27 | 303 ± 23 | 286 ± 23* |
| 7 | 318 ± 30 | 313 ± 27 | 312 ± 26 | 294 ± 27* |
| 14 | 327 ± 29 | 324 ± 26 | 319 ± 24 | 302 ± 26* |
| 21 | 315 ± 29 | 311 ± 24 | 308 ± 20 | 297 ± 22* |
| Wt. Gain 0-21 | 21 | 22 | 24 | 35 |

[a]Data were extracted from Study No. 100647, pp. 128, 129, 131, 132, 136, 137, 139, and 140.

[b]Standard deviation for weight gain was not provided.

*Significantly different from control ($p \leq 0.05$)

Table 4. Effects of Dietary Administration of Imidacloprid on $F_0$ Reproductive Parameters, Offspring Survival, and $F_{1A}$ Pup Body Weight[a]

| Parameter | Dietary Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 250 | 700 |
| No. matings ($F_0$ parents) | 29 | 28 | 30 | 30 |
| Mating index (%)[b] | 100 | 97 | 100 | 100 |
| Fertility index (%)[c] | 100 | 97 | 93 | 97 |
| Gestation index (%)[d] | 100 | 100 | 100 | 100 |
| Gestation length (days) | 22.2 | 22.1 | 22.3 | 22.1 |
| No. females with liveborn pups | 29 | 28 | 28 | 29 |
| Total no. live pups | | | | |
| Day 0 | 310 | 294 | 325 | 310 |
| Day 4 precull | 299 | 277 | 313 | 299 |
| Day 21 | 207 | 206 | 214 | 203 |
| Mean no. live pups/litter | | | | |
| Day 0 | 10.7 | 10.5 | 11.6 | 10.7 |
| Day 4 precull | 10.3 | 9.9 | 11.2 | 10.3 |
| Day 21 | 7.1 | 7.4 | 7.6 | 7.0 |
| Live birth index (%)[e,h] | 99 | 99 | 98 | 99 |
| Viability index (%)[f,h] | 96 | 94 | 96 | 96 |
| Lactation index (%)[g,h] | 92 | 94 | 96 | 89 |
| Mean pup body weight (g) | | | | |
| Day 0 | 5.5 | 5.6 | 5.6* | 5.6* |
| Day 7 | 13.8 | 13.6 | 14.1 | 12.5* |
| Day 21 | 47.1 | 45.5* | 46.6 | 40.8* |
| Sex ratio (% males day 0) | 51 | 50 | 50 | 51 |

[a]Data were extracted from Study No. 100647, pp 161, 162, 171, 185, 193-200, 224, and 240

[b]Mating index: No. of mated females expressed as % of No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]Live birth index: Percentage of pups born alive based on No. of total pups born

[f]Viability index: Percentage of pups surviving four days based on No. of pups on day 1

[g]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

[h]Calculated by the reviewers; not statistically analyzed

*Significantly different from control ($p \leq 0.05$)

Table 5. Effects of Dietary Administration of Imidacloprid on $F_0$ Reproductive Parameters, Offspring Survival, and $F_{1B}$ Pup Body Weight[a]

| Parameter | Dietary Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 250 | 700 |
| No. matings ($F_0$ parents) | 29 | 29 | 30 | 30 |
| Mating index (%)[b] | 100 | 100 | 100 | 100 |
| Fertility index (%)[c] | 93 | 93 | 93 | 90 |
| Gestation index (%)[d] | 100 | 100 | 100 | 100 |
| Gestation length (days) | 22.0 | 22.1 | 22.1 | 22.1 |
| No. females with liveborn pups | 27 | 27 | 28 | 27 |
| Total no. live pups | | | | |
| Day 0 | 320 | 303 | 313 | 283 |
| Day 4 precull | 315 | 297 | 306 | 275 |
| Day 21 | 199 | 212 | 214 | 193 (26)[e] |
| Mean no. live pups/litter | | | | |
| Day 0 | 11.9 | 11.2 | 11.2 | 10.5 |
| Day 4 precull | 11.7 | 11.0 | 10.9 | 10.2 |
| Day 21 | 7.4 | 7.9 | 7.6 | 7.4 (26)[e] |
| Live birth index (%)[f,i] | 100 | 99 | 98 | 99 |
| Viability index (%)[g,i] | 98 | 98 | 98 | 97 |
| Lactation index (%)[h,i] | 95 | 99 | 97 | 90 |
| Mean pup body weight (g) | | | | |
| Day 0 | 5.6 | 5.8[*] | 5.8[*] | 5.8[*] |
| Day 7 | 14.6 | 15.2[*] | 15.3[*] | 14.0[*] |
| Day 21 | 49.8 | 50.2 | 49.7 | 45.0[*] |
| Sex ratio (% males day 0) | 45 | 45 | 45 | 46 |

[a]Data were extracted from Study No. 100647, pp 163, 164, 174, 186, 201-208, 225, and 241

[b]Mating index: No. of mated females expressed as % of No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]No. of litters

[f]Live birth index: Percentage of pups born alive based on No. of total pups born

[g]Viability index: Percentage of pups surviving four days based on No. of pups on day 1

[h]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

[i]Calculated by the reviewers; not statistically analyzed

[*]Significantly different from control ($p \leq 0.05$)

Table 6. Effects of Dietary Administration of Imidacloprid on $F_1$ Reproductive Parameters, Offspring Survival, and $F_{2A}$ Pup Body Weight[a]

| Parameter | Dietary Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 250 | 700 |
| No. matings ($F_1$ parents) | 26 | 26 | 26 | 26 |
| Mating index (%)[b] | 100 | 100 | 100 | 100 |
| Fertility index (%)[c] | 85 | 89 | 85 | 96 |
| Gestation index (%)[d] | 100 | 100 | 100 | 100 |
| Gestation length (days) | 22.4 | 22.3 | 22.3 | 22.3 |
| No. females with liveborn pups | 22 | 23 | 21 | 25 |
| Total no. live pups | | | | |
| Day 0 | 222 | 254 | 197 | 239 |
| Day 4 precull | 202 (21)[e] | 243 | 189 | 234 |
| Day 21 | 148 | 172 | 141 | 177 |
| Mean no. live pups/litter | | | | |
| Day 0 | 10.1 | 11.0 | 9.4 | 9.6 |
| Day 4 precull | 9.6 (21)[e] | 10.6 | 9.0 | 9.4 |
| Day 21 | 7.0 | 7.5 | 6.7 | 7.1 |
| Live birth index (%)[f,j] | 97 | 99 | 98 | 100 |
| Viability index (%)[g,j] | 91 | 96 | 96 | 98 |
| Lactation index (%)[h,j] | 99 | 99 | 95 | 99 |
| Mean pup body weight (g) | | | | |
| Day 0 | 5.8 | 5.6 | 5.7 | 5.7 |
| Day 7 | 15.0 | 14.9 | 14.4* | 14.1* |
| Day 21 | 44.3 | 44.3 | 43.6 | 40.3* |
| Sex ratio (% males day 0) | 51 | 53 | 46 | 43 |

[a]Data were extracted from Study No. 100647, pp 165, 166, 181, 187, 209-215, 226, and 242

[b]Mating index: No. of mated females expressed as % of total No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]No. of litters

[f]Live birth index: Percentage of pups born alive based on No. of total pups born

[g]Viability index: Percentage of pups surviving four days based on No. of pups on day 1

[h]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

[j]Calculated by the reviewers; not statistically analyzed

*Significantly different from control ($p \leq 0.05$)

Table 7. Effects of Dietary Administration of Imidacloprid on $F_1$ Reproductive Parameters, Offspring Survival, and $F_{2B}$ Pup Body Weight[a]

| Parameter | Dietary Level (ppm) | | | |
|---|---|---|---|---|
| | 0 | 100 | 250 | 700 |
| No. matings ($F_1$ parents) | 25 | 26 | 26 | 26 |
| Mating index (%)[b] | 96 | 100 | 100 | 100 |
| Fertility index (%)[c] | 92 | 77 | 100 | 100 |
| Gestation index (%)[d] | 100 | 100 | 100 | 100 |
| Gestation length (days) | 21.9 | 21.9 | 22.0 | 21.3 |
| No. females with liveborn pups | 24 | 20 | 26 | 26 |
| Total no. live pups | | | | |
| Day 0 | 260 | 202 | 229 | 278 |
| Day 4 precull | 254 | 198 | 222 (25)* | 273 |
| Day 21 | 180 | 146 | 172 | 185 |
| Mean no. live pups/litter | | | | |
| Day 0 | 10.8 | 10.1 | 9.2 | 10.7 |
| Day 4 precull | 10.6 | 9.9 | 8.9 (25)* | 10.5 |
| Day 21 | 7.5 | 7.3 | 6.9 | 7.1 |
| Live birth index (%)[f,i] | 98 | 99 | 97 | 99 |
| Viability index (%)[g,i] | 98 | 98 | 97 | 98 |
| Lactation index (%)[h,i] | 98 | 97 | 97 | 94 |
| Mean pup body weight (g) | | | | |
| Day 0 | 5.9 | 5.8 | 5.5 | 5.3 |
| Day 7 | 15.6 | 15.5 | 15.1 | 14.2* |
| Day 21 | 50.7 | 50.5 | 48.7* | 46.9* |
| Sex ratio (% males day 0) | 48 | 52 | 46 | 50 |

[a]Data were extracted from Study No. 100647, pp 167, 168, 184, 188, 216-222, 227, and 243

[b]Mating index: No. of mated females expressed as % of total No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]No. litters

[f]Live birth index: Percentage of pups born alive based on No. of total pups born

[g]Viability index: Percentage of pups surviving four days based on No. of pups on day 1

[h]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

[i]Calculated by the reviewers; not statistically analyzed

*Significantly different from control ($p \leq 0.05$)

Reviewed by: Myron S. Ottley, Ph.D. MSO
Section IV, Tox Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 7/22/93
Section IV, Tox Branch I (H7509C)

# DATA EVALUATION REPORT—Supplemental
(Main DER NED Doc# 007960)

| | |
|---|---|
| STUDY TYPE: | **Developmental Toxicity—Rabbit** (83-3) |
| TOX. CHEM. NO.: | 497E |
| PC NUMBER: | 129099 |
| MRID NO.: | 422563-39 |
| TEST MATERIAL: | **NTN 33893 Technical** |
| SYNONYMS: | Imidacloprid (proposed)<br>1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine |
| STUDY NUMBER: | 083518 |
| SPONSOR: | Miles Corp. |
| TESTING FACILITIES: | RCC, Research and Consulting Company AG, Switzerland<br>RCC, UMWELTCHEMIE AG, Switzerland |
| TITLE OF REPORT: | Embryotoxicity Study (Including Teratogenicity) with NTN 33893 Technical in the Rabbit |
| AUTHOR: | H. Becker, K. Biedermann |
| REPORT ISSUED: | January 8, 1992 |

## CONCLUSIONS

NTN 33893 Technical was administered by gavage to Chinchilla rabbits at 0, 8, 24 or 72 mg/kg/d during gestation days 6 - 18.

**Maternal**
NOEL =24 mg/kg/d
LOEL =72 mg/kg/d: decreased body weight, increased resorption, increased abortion, and possibly increased death)

**Developmental**
NOEL = 24 mg/kg/d
LOEL = 72 mg/kg/d (Increased resorptions, decreased body weight, increased skeletal abnormalities)

CLASSIFICATION: **Core Minimum**
This study satisfies the guideline (83-3) requirements for a developmental toxicity study, and is acceptable for regulatory purposes.

## DISCUSSION

During the HED RfD Committee meeting on April 22, 1993, it was felt that the data presented on the developmental toxicity of NTN 33893 Technical did not support the conclusions. Specifically, it was questioned whether the increased resorptions seen at the high dose level were treatment-related or merely due to normal variation.

In a subsequent meeting with the Developmental Toxicity Subcommittee, the Submitter's data were reexamined, and the Subcommittee concluded that the conclusions of the original DER were correct. The Subcommittee requested that the attached table be included as part of the DER since it more adequately supported the conclusions made in the DER than other tables which were included.

The attached table, taken from page 44 of the study report, shows clearly that post-implantation loss was significantly ($p \leq 0.05$) higher than controls (10.8% vs. 4.2%). This table replaces the table in the original DER which was taken from page 46 of the study report.

As stated before, the NOEL and LOEL are not changed.

Toxicology Review # 010537 9/3/93

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Page 13 is not included in this copy.

Pages _____ through _____ are not included.

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The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

√ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

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010537

# FINAL

DATA EVALUATION REPORT

IMIDACLOPRID

Study Type: Metabolism

Prepared for:

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

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Pia Lindstrom Date 3/31/93
Pia Lindstrom, DVM

Independent Reviewer Karen M Gan Date 3/31/93
Karen Gan, M.S.

QA/QC Manager William L McLellan for Date 3/31/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-66
Clement Numbers: 180 - 186
Project Officer: Caroline Gordon

GUIDELINE SERIES 85-1: Metabolism

EPA Reviewer: Paul Chin, Ph.D. Signature: Paul Chin
Review Section II, Toxicology Branch I/HED Date: 4/8/93

EPA Section Head: Marion Copley, D.V.M. Signature: Marion Copley
Review Section IV, Toxicology Branch I/HED Date: 4/9/93

DATA EVALUATION REPORT

STUDY TYPE: Metabolism in rats; Guideline Series 85-1

EPA IDENTIFICATION NUMBERS

Tox. Chem. Number: 497E

EPA P.C. Code: 129099

MRID Numbers:
Part a) 422563-54
Part b) 422563-56
Part c) 422563-57
Part d) 422563-73
Part e) 422563-59
Part f) 422563-58
Part g) 422563-55

TEST MATERIAL: Imidacloprid

Part a) [Methylene-$^{14}$C] Imidacloprid
Part b) [Methylene-$^{14}$C] Imidacloprid
Part c) [Imidazolidine-4,5-$^{14}$C] Imidacloprid
Part d) [Methylene-$^{14}$C] Imidacloprid and [Methylene-$^{14}$C] WAK 3839
Part e) Imidacloprid
Part f) Imidacloprid
Part g) [Methylene-$^{14}$C] Imidacloprid

SYNONYM: 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine]; NTN 33893™

SPONSOR: Parts a, b, c, and g) Miles Inc., Stilwell, KS
Parts d, e, f) Mobay Corporation, Stilwell, KS

TESTING FACILITY: Parts a, b, c, d, and g) Bayer AG, Leverkusen-Bayerwerk, Germany
Parts e and f) Mobay Corporation, Stilwell, KS

AUTHORS:
Part a) O. Klein and W. Karl
Parts b, d, and g) O. Klein
Part c) O. Klein and A Brauner
Part e) K.D. Moore
Part f) K.D. Moore

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nicotinic acid and its mercapturic acid derivative. The second route included the hydroxylation followed by elimination of water of the parent compound rendering NTN 35884.

A comparison between [methylene-$^{14}$C]-Imidacloprid and [imidazolidine-4,5-$^{14}$C]-Imidacloprid showed that, while the rate of excretion was similar, the renal portion was higher with the imidazolidine-labeled compound. In addition, accumulation in tissues was generally higher with the imidazolidine-labeled compound.

A comparison between Imidacloprid and one of its metabolites, WAK 3839, showed that the total elimination was the same for both compounds. The proposed metabolic pathways for these two compounds were different. WAK 3839 was formed following pretreatment (repeated dosing) of Imidacloprid.

CORE CLASSIFICATION: Acceptable. These studies meet the requirements set forth under Guideline Series 85-1 for a metabolism study in rats.

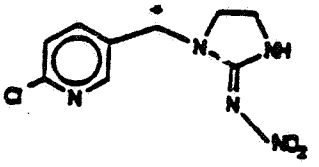
MATERIALS

Parts a and b

Imidacloprid

| | |
|---|---|
| Purity: | 99.9% |
| Contaminants: | None reported |
| Description: | Colorless crystals |
| Radiochemical purity: | >99% by HPLC and TLC |
| Specific activity: | 150.7 μCi/mg |
| Batch numbers: (non-labeled compounds) | APF 08128650 and 980208ELB01 |

Chemical structure:



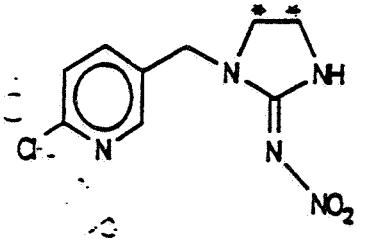

* denotes [$^{14}$C] labeling

Part c

Imidacloprid

| | |
|---|---|
| Purity: | >99% |
| Contaminants: | None reported |
| Description: | Colorless crystals |
| Radiochemical purity: | >99% |
| Specific activity: | 124 µCi/mg (low dose)<br>0.827 µCi/mg (high dose) |
| Batch numbers: (labeled compounds) | KML 16094 (low dose)<br>890315ELB01 (high dose) |

Chemical structure:



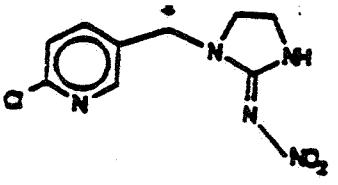
* denotes [$^{14}$C] labeling

Part d

Imidacloprid

| | |
|---|---|
| Purity: | >99% |
| Contaminants: | None reported |
| Description: | Solids |
| Radiochemical purity: | >99%; 98.4% |
| Specific activity: | 86.4 µCi/mg (low dose)<br>91.8 µCi/mg (high dose)<br>123 µCi/mg (pretreatment) |
| Batch numbers: (labeled compounds) | KML 1417, KML 1705 |

Chemical structure:



* denotes [$^{14}$C] labeling

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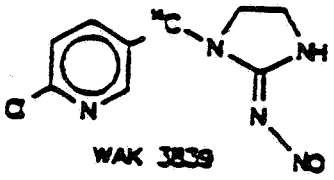
WAK 3839

| | |
|---|---|
| Purity: | >99% |
| Contaminants: | None reported |
| Description: | Solids |
| Radiochemical purity: | WAK 3839, 97.8-98.5% |
| Specific activity: | 40.2 μCi/mg (low dose)<br>50.43 μCi/mg (isotope dilution rat)<br>128.8 μCi/mg (isotope dilution mouse) |
| Batch numbers: (labeled compounds) | TSH 3520, TSH 3550, TSH 3552 |

Chemical structure:




WAK 3839

* denotes [$^{14}$C] labeling

Vehicle (all studies): Physiological saline or 0.5% Tragacanth

Test Animals (all studies)

| | |
|---|---|
| Species: | Rat |
| Strain: | Wistar BOR: WISW (SPF Cpb) |
| Source: | Winkelmann Versuchstierzucht GmbH & Co., Borchen, Germany |
| Age: | Not reported |
| Weight: | Approximately 200 g (individual data not submitted) |

Animal husbandry (all studies)

Acclimation time was not reported. During the excretion studies, animals were housed in metabolism cages to allow for collection of the excreta; during other studies, animals were housed in plastic cages on wood shavings. Each animal was given 15 g of food per day (Altromin 1324 Standard Food, 4937 Lage, Germany). Water was available ad libitum throughout the study. During the nonradioactive pretreatment period and bile-fistulation portion of the studies, temperature and humidity were controlled at 20°C and 40%-80%, respectively. It was stated that during the test period, the animals were kept at room temperature; however, temperature and humidity were not specified.

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Study Design

These studies were designed to assess the absorption, distribution, metabolism, and excretion of Imidacloprid when administered intravenously or via oral gavage to rats; when labelled at different molecular sites; and when compared to its metabolite, WAK 3839 (Parts a-d). Also, acute versus chronic exposure was compared as well as species differences following chronic exposure in the diet (Part d).

Group arrangement

Animals were randomized by lot for all experiments. The following experiments/groups were designed for the various investigative parts.

Parts a and b: Studies were designed to evaluate distribution, metabolism, and excretion of Imidacloprid, when administered intravenously or orally (single and repeated dosing studies).

| Studies | Dose | Males | Females |
|---|---|---|---|
| Expired $CO_2$ experiment | 20.0 mg/kg (oral) | 5 | -- |
| Low-dose experiment | 1.0 mg/kg (i.v.) | 5 | 5 |
| Low-dose experiment | 1.0 mg/kg (oral) | 5 | 5 |
| High-dose experiment | 20.0 mg/kg (oral) | 5 | 5 |
| Chronic low-dose experiment[a] | 1.0 mg/kg (oral) | 5 | 5 |
| Bile-fistulation experiment[b] | 1.0 mg/kg (intraduodenal) | 5 | -- |
| High-dose experiment[c] | 20.0 mg/kg (oral) | 5[d] | -- |

[a]Nonradiolabeled 1.0 mg/kg/day for 14 days followed by radiolabeled 1.0 mg/kg/day on day 15

[b]Experiment conducted to quantify the absorbed amount of the total radioactivity and to determine the rate and extent of biliary excretion.

[c]Experiment conducted to fulfill Japanese MAFF requirements.

[d]Four groups of 5 animals per group were killed after 40 minutes, 1.5, 3, and 6 hours and tissue accumulations were evaluated.

Part c: Studies were designed to identify and compare metabolites of [methylene-$^{14}$C]-labelled Imidacloprid versus [imidazolidine-4,5-$^{14}$C]- labelled Imidacloprid when administered orally to rats.

| Studies | Dose | Males | Females |
|---|---|---|---|
| Expired $CO_2$ experiment | 1.0 mg/kg | 5 | -- |
| Low-dose experiment | 1.0 mg/kg | 5 | 5 |
| High-dose experiment | 150.0 mg/kg | 5 | -- |

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Part d: Studies were designed to identify and compare metabolites of [methylene-$^{14}$C]-labelled Imidacloprid versus [methylene-$^{14}$C]-labelled WAK 3839 (a metabolite of Imidacloprid) when administered orally (including non-radioactive pretreatment) to rats.

| Studies | Volume | Dose | Males |
|---|---|---|---|
| Imidacloprid low-dose | 10 mL | 1.0 mg/kg | 5 |
| WAK 3839 | 10 mL | 1.0 mg/kg | 5 |
| Imidacloprid high-dose | 10 mL | 150.0 mg/kg | 7 |
| Imidacloprid chronic[a] | 2 mL | 80.0 mg/kg | 10 |

[a]Animals were pretreated with a diet containing 1800 ppm unlabelled Imidacloprid for one year then received an oral dose of $^{14}$C-Imidacloprid.

Dosing Solutions (all studies)

For oral administration, the test material was administered in a volume of 10 mL/kg. For intraduodenal administration, the volume was 1 mL/kg. For i.v. administration, the volume was not specified. Test solutions, both labeled and unlabeled, were prepared by dissolving the test substance in physiological saline using an ultrasonic water bath at 70°C or by suspending the test substance homogeneously in 0.5% Tragacanth. Solutions were prepared immediately prior to administration with the exception of the nonradioactive portion of the chronic low-dose experiment, in which doses to be administered over the weekend were prepared the preceding Friday. The solutions were stable for 48 hours as demonstrated by thin layer chromatography. Methods for quantifying Imidacloprid concentrations in aqueous dose mixtures and in liquid aerosol atmosphere of inhalation chambers were described separately (MRID Nos. 422563-59 and 422563-58; Parts e and f, respectively).

Sample Collection (all studies)

Urine samples were collected for intervals 0-4, 4-8, 8-24, and 24-48 hours following dosing (deviations from these intervals are indicated in the tables). The cage rinsing solutions were collected in the urine containers. Radioactivity in urine, extracts, and solutions was determined by liquid scintillation counting (LSC).

Feces were collected for intervals 0-24 and 24-48 hours following dosing. Samples were lyophilized and homogenized prior to extraction, and then prepared for LSC.

Tissues were collected after 48 hours and included: plasma, erythrocytes, spleen, gastrointestinal tract, liver, bile, kidney, testis, muscle, bone, heart, lung, brain, skin, uterus, ovary, and renal fat. They were lyophilized and combusted in an oxygen atmosphere before radioactive determination. Radioactivity in both solids and liquid samples were determined by LSC.

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Metabolite Analysis

Parts a and b: Major metabolites were isolated from the 6-hour urines and feces by HPLC. Minor metabolites were isolated from the 24-hour urines and feces. Five major and four minor fractions were obtained and further purified and analyzed by spectroscopic methods (e.g., gas chromatography, mass spectrometry, and/or nuclear magnetic resonance spectroscopy). Metabolites were identified with reference compounds.

Part c: Metabolites were isolated from the high-dose 0-24-hour urine sampling. Following several purification procedures, two peak groups appeared, which were further analyzed by NMR and mass spectroscopy. Metabolite analyses were not conducted on fecal samples since the fecal elimination route consisted of only 6%-8% of the administered dose.

Part d: Metabolites in urine were identified using renal samples from the 0-4 and 4-24-hour intervals combined and the alkaline solvent system for quantification. Metabolites in feces were identified using fecal samples from the 0-24-hour intervals which were lyophilized, extracted with water, and then further purified before being chromatographed.

Compliance

- Statements of No Data Confidentiality Claims, signed and dated, were provided.
- Statement of Compliance with EPA, OECD, and/or MAFF GLPs, signed and dated, were provided.
- Statements of Quality Assurance, signed and dated, were provided.

RESULTS

ELIMINATION AND RECOVERY

Parts a and b: More than 90% of the administered radioactivity was recovered in the tissues and excreta within 48 hours postexposure after oral and i.v. dosing (Tables 1 and 2). No biologically significant differences were observed between the sexes or with regard to route of administration and dose level. A slight difference in excretion of Imidacloprid was observed between males and females at the high-dose. The females excreted more of the radioactivity via urine than did males (Table 2). After 24 hours, renal elimination accounted for approximately 67%-78% of excreted radioactivity in all groups, while fecal elimination accounted for approximately 16%-24% in males and females (Table 1). After 48 hours, these values had increased to 69%-80% and 17%-25%, respectively. The tissues accounted for approximately 0.5% of the radioactivity (Table 2). For both sexes, irrespective of route of administration and dose level, the major sites for radioactive accumulation were liver, kidney, lung, skin, and plasma; the minor sites were brain and testis (Appendices I and II). Time was not a significant factor when considering tissue distribution of radioactivity.

The 'expired air'-experiment demonstrated that no significant amount of radioactivity was expired in male rats over 48 hours (Table 2), thus indicating that the labeling position within the molecule was stable under *in vivo* conditions.

The 'bile-fistulated'-experiment demonstrated that the major part of the fecal radioactivity originated in the bile (Table 2). Bile-fistulated animals excreted 5% in the feces versus 37% in the bile.

Part c: In the metabolism and biokinetic parts of the imidazolidine-4,5-$^{14}$C-labeled Imidacloprid studies, >99% of the administered radioactivity was also recovered within 48 hours in all groups (Table 3). Again, no biologically significant differences were observed between the sexes or with regard to dose level. After 24 hours, renal elimination accounted for approximately 76%-93% of excreted radioactivity, while fecal elimination accounted for 4%-8% (Table 3). After 48 hours, these values had increased to 90%-94% for renal elimination and 6%-8% for fecal elimination. The tissues accounted for approximately 1% of the radioactivity (Table 3). For both sexes, irrespective of dose level, the major sites of radioactive accumulation were liver, kidney, lung, and skin (Appendix III); the minor sites were brain and muscle. In addition, the female values were slightly lower than the male values.

The 'expired air'-experiment (data not shown) demonstrated that no significant amount of radioactivity (0.111% of recovered radioactivity) was expired in male rats over 48 hours. This indicated that the labeling position within the molecule was stable under *in vivo* conditions.

Part d: In the comparison of methylene-labelled Imidacloprid and WAK 3839 (see page 6 for the chemical structure), no significant differences were noted in the absorption, distribution, and excretion of the total radioactivity. The renal/fecal elimination rate was ≈3:1. Forty-eight hours after oral administration of 1 mg/kg Imidacloprid and 1 mg/kg WAK 3839, 77% and 73% of the given dose, respectively, were eliminated via urine, while 21% and 14%, respectively, were eliminated via feces (Table 4). The final amount was similar for both compounds. Fecal elimination was almost complete within 24 hours for both compounds. In general, the pretreatment group showed similar renal and fecal excretion patterns as single dose groups.

Although more radioactivity was found in the tissues of the Imidacloprid animals, total body accumulation was <1% for both compounds at the low-dose level (Table 4). In the Imidacloprid high-dose group, 3% of administered radioactivity was recovered. Main sites of accumulation included skin, lung, liver, and kidney for Imidacloprid animals, while it included lung, renal fat, liver and kidney for WAK 3839 animals (Appendix IV). For both compounds, testis and brain were the minor sites of accumulation.

Part g: In a separate whole-body radiography study, it was further demonstrated that tissue distribution of Imidacloprid was not time-dependent. In this qualitative experiment, male rats were investigated 1, 4, 8, and 48 hours after oral administration or 5 minutes after i.v. administration of 20 mg/kg methylene-labeled Imidacloprid (data not shown). The relative tissue distribution did not change significantly over time, although the amount of radioactivity rapidly diminished. As estimated from the radiograph, within

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the first hour, major accumulation of radioactivity was detectable in the liver, kidney, adrenal, muscle, skin, walls of the aorta, stomach, and small intestine, connective tissue attached to the spinal cord, and salivary, cowperian, and thyroid glands. Minor accumulation was noted in the lungs, fat, brain, testis, and the mineral part of the bones. After 24 hours, no radioactivity was observed in the stomach, but radioactivity was accumulating in the intestine. After 48 hours, the only tissues above the detection limit were skin, nasal mucosa, liver, kidney, thyroid, walls of the aorta, and the connective tissue attached to the spinal cord. Overall, these results were in agreement with the quantitative tissue distribution studies.

PHARMACOKINETICS

Part b: For methylene-labeled Imidacloprid, plasma curve analysis demonstrated that the compound was absorbed immediately with <2.5 minutes of calculated lagtime for all dose groups. The maximum relative concentration in plasma was between 1.1 and 2.5 hours. The compound and its metabolites were easily distributed into peripheral compartments as demonstrated by the distribution volume under steady state conditions (roughly equal). The average distribution half-life was 35 minutes. Elimination half-lives (calculated from two exponential terms) were 3 and 26-118 hours.

Part c: For imidazolidine 4,5-$^{14}$C-labeled Imidacloprid, one hour after low-dose administration and 4 hours after high-dose administration, maximum plasma concentrations were reached in males; 1.5 hours after low-dose administration it was reached in females. Terminal elimination half-lives were dose dependent. They were 21.34 hours (females) and 24.89 hours (males) for low-dose animals and 9.04 hours for high-dose males (females not evaluated). The mean residence time was longer for the high-dose animals (low-dose: 9.0 hours [males] and 8.56 hours [females]; high-dose: 14.25 hours). Consequently, the renal excretion rate was slightly slower, although the same amount of radioactivity was eliminated in the urine at the end of the test period.

Part d: In the studies comparing methylene-labeled Imidacloprid and WAK 3839, the time-course of the plasma levels were similar for both Imidacloprid and WAK 3839. Absorption started immediately and estimated distribution half-lives were ≈22 minutes. The two compounds differed with regard to maximum plasma concentration of the radioactivity (0.77 hours for WAK 3839; 1.16 hours for Imidacloprid). Significant differences were observed in the pharmacokinetic basic parameters, in total and renal clearance, and in distribution volume at steady state. No significant differences were noted in terminal half-lives, areas under the curves, and mean residence times.

METABOLISM

Part a: In the metabolism part of the methylene-labeled Imidacloprid studies, identified metabolites were found in both sexes and all dose groups. No biologically significant differences were observed in the pattern of excretion with regard to sex, dose level, and route of administration. Two major routes of biotransformation were evident. The first route included an

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oxidative cleavage of the parent compound rendering 6-chloronicotinic acid and its glycine conjugate. Dechlorination of this metabolite formed the 6-hydroxy nicotinic acid and its mercapturic acid derivative. The second route included the hydroxylation of imidazolidine followed by elimination of water of the parent compound rendering NTN 35884. A summary of results is presented in Tables 5 and 6. The proposed degradation pathway is presented in Figure 1.

Urinary metabolites (Table 5) consisted of WAK 3583 (males: 17%-28%; females: 19%-24%), 5-OH-Imidacloprid (WAK 4103) (males: 16%-18%; females: 15%-16%), NTN 35884 (males: 9%-13%; females: 8%-9%), chloronicotinic acid (males: 4%-8%; females: 3%-8%), and the parent compound, Imidacloprid (males: 9%-14%; females: 11%-15%). A glycine conjugate of 6-S-$CH_3$-nicotinic acid appeared as a minor metabolite, accounting for 2%-6% (males and females) of the recovered radioactivity. NTN 33823 was not detected in the urine.

Fecal metabolites (Table 6) consisted of NTN 33823 (males and females: 2%-3%), glycine conjugate of 6-S-$CH_3$-nicotinic acid (males and females: 1%-2%), NTN 35884 (males and females: 1%-2%), and the parent compound, Imidacloprid (males and females: 1%-2%). WAK 3583, WAK 4103, and chloronicotinic acid were not detected in the feces.

There were minor differences between dose and sex groups. The amount of unchanged parent compound in excreta was slightly higher after i.v. low-dosing than after oral low-dosing. Males formed slightly more 6-chloro-nicotinic acid and WAK 3583 in the urine than did females after iv or oral low-dosing. Nonradioactive pretreatment in animals produced less WAK 3583 and more 6-chloronicotinic acid formation in both sexes as compared to single oral low-dosing. The amount of unchanged parent compound was higher and the amount of NTN 35884 was lower in excreta of females than of males after oral high-dosing.

Part c: No significant differences were observed in the pattern of excretion of various metabolites with regard to sex. However, there was a dose-related difference and a metabolic saturation may have occurred after high oral dosing. In the high-dose males, the amount of KNO 0523 was reduced to 19% and the amount of NTN 33968 and the parent compound was increased to 15% and 14%, respectively, as compared to the low-dose males. Urinary metabolites at 24 hours after oral administration of [imidazolidine-4,5-$^{14}$C]-Imidacloprid is presented in Table 7.

Renal metabolites included KNO 0523, NTN 33968, WAK 4103, NTN 35884, and the parent compound. Together, they constituted 73%-83% of the recovered urinary radioactivity. An unidentified metabolite accounted for 10%-18% of the radioactivity. Due to its linkage to very polar matrix components, this metabolite could not be identified but this metabolite should comprise the imidazolidine moiety only. In addition, the urinary metabolites of Imidacloprid were already completely identified by the previous analysis of fecal metabolites of [methylene-$^{14}$C] Imidacloprid.

Fecal metabolites were not identified since they constituted only 6.2%-11.2% of recovered radioactivity. In addition, the previous analysis of fecal metabolites of [methylene-$^{14}$C] Imidacloprid identified more than 50% of the fecal metabolites.

A comparison between the two differently labeled Imidacloprid compounds (Tables 8) showed that, while the rate of excretion was similar, the renal portion was higher with the imidazolidine-labeled compound (90% versus 75% with the methylene-labeled compound; see Tables 2 and 3). In addition, accumulation of radioactivity in tissues was low (<1% of administered dose) and, in general, higher with the imidazolidine-labeled compound. A qualitative comparison with regard to metabolites demonstrated that, as long as the metabolites contained both heterocycles, they rendered the same kind of metabolites (i.e., parent compound, WAK 4103, and NTN 35884). A quantitative comparison between these same metabolites (Table 8) demonstrated that they were of the same order regardless of labeling moiety. The proposed degradation pathway is presented in Figure 2.

Part d: In the studies comparing methylene-labeled Imidacloprid and WAK 3839, the identification of renal and fecal metabolites in the low-dose groups demonstrated different metabolic pathways (Tables 9 and 10). The following metabolites in urine and feces were found in Imidacloprid animals after 24 hours postexposure: 6-chloronicotinic acid (7% of total recovered radioactivity); WAK 3583 (25%); NTN 35884 (10%); WAK 3839 (0.5%); WAK 4103 (14%); and unchanged parent compound (11%). In the WAK 3839 animals, only unchanged parent compound (63%) and NTN 33823 (6%) were found in the excreta after 24 hours postexposure.

WAK 3839 formed at a higher rate during chronic feeding of Imidacloprid. The amount of WAK 3839 excreted in the urine of single-dosed rats was comparative to the trace impurity contained in the radioactive batch of Imidacloprid indicating no *in vivo* formation of this metabolite (Table 9). However, the amount of WAK 3839 excreted in the pretreated rats showed an increasing amount from 1% at 0-7 hours to 17% at 24-48 hours (Table 9), which is further supported by the findings of the isotope analysis in urine from chronically fed rats and mice. Rats and mice were fed 1800 and 2000 ppm of Imidacloprid respectively, for one year, then received a single oral dose of [methylene-$^{14}$C]-WAK 3839 (Study Nos. T303005 and T4029986). The concentrations of WAK 3839 were estimated to be 9 and 1.5 mg/100 mL urine in rats and mice, respectively. The proposed metabolic pathways are presented in Figure 3.

## REVIEWERS' DISCUSSION/CONCLUSIONS

The methylene-labelled Imidacloprid was rapidly absorbed following a calculated lagtime of <2.5 minutes and eliminated in the excreta (90% of the dose within 24 hours; 96% within 48 hours) demonstrating no biologically significant differences between sexes, dose levels, or route of administration. Elimination was mainly renal (70%-80%) with less contribution from feces (17%-25%). The major part of the fecal radioactivity originated in the bile. Total body accumulation after 48 hours constituted only 0.5% of the radioactivity with the liver, kidney, lung, skin, and plasma being the major sites of accumulation. Maximum plasma concentration was reached between 1.1 and 2.5 hours. Elimination half-lives were 3 and 26-118 hours. Two major routes of biotransformation were evident. The first route included an oxidative cleavage of the parent compound rendering 6-chloronicotinic acid and its glycine conjugate. Dechlorination of this metabolite formed the 6-hydroxy nicotinic acid and its mercapturic acid derivative. The second route included

the hydroxylation followed by elimination of water of the parent compound rendering NTN 35884.

A comparison between [methylene-$^{14}$C] Imidacloprid and [imidazolidine-4,5-$^{14}$C] Imidacloprid demonstrated that, while the rate of excretion was similar, the renal portion was higher with the imidazolidine-labeled compound (90% versus 75% with the methylene-labeled compound). In addition, accumulation in tissues was generally higher with the imidazolidine-labelled compound. As long as the metabolites contained both heterocycles, they rendered the same kind of metabolites (parent compound, WAK 4103, and NTN 35884).

A comparison between Imidacloprid and one of its metabolites, WAK 3839, showed that the renal elimination of WAK 3839 was faster although the total elimination was the same for both compounds (Part d). The proposed metabolic pathways for these two compounds were different. WAK 3839 was formed following pretreatment (repeated dosing) of Imidacloprid.

These studies have been classified as Acceptable based on well executed designs and more than adequate data reporting. Every study (reported and referred to) has been described in adequate detail and fulfilled the Guideline requirements. However, the overall data reporting would have greatly benefited from an overall summary of study designs, strategies, results, and conclusions.

TABLE 1. Percent Recovery of Administered Radioactivity After I.V. or Oral Administration of Imidacloprid (Parts a and b)[a]

| Fraction | Dose Group (% of Administered Dose) i.v. 1 mg/kg | p.o. 1 mg/kg | p.o. 1 mg/kg pretreatment | p.o. 20 mg/kg |
|---|---|---|---|---|
| | | Males | | |
| URINE | | | | |
| 0 to 4 hours | 26.18 | 22.52 | 13.20 | 28.86 |
| 0 to 8 | 61.26 | 56.77 | 44.70 | 56.06 |
| 0 to 24 | 72.72 | 71.23 | 67.32 | 72.15 |
| 0 to 48 | 73.43 | 72.57 | 69.04 | 73.26 |
| FECES | | | | |
| 0 to 24 hours | 18.83 | 19.63 | 21.90 | 20.79 |
| 0 to 48 | 19.34 | 20.26 | 23.83 | 21.25 |
| TOTAL RECOVERY | 92.78 | 92.83 | 92.87 | 94.51 |
| | | Females | | |
| URINE | | | | |
| 0 to 4 hours | 27.27 | 29.78 | 19.30 | 27.39 |
| 0 to 8 | 52.51 | 55.86 | 38.86 | 50.41 |
| 0 to 24 | 70.79 | 70.76 | 69.64 | 77.81 |
| 0 to 48 | 72.53 | 72.42 | 71.83 | 79.59 |
| FECES | | | | |
| 0 to 24 hours | 16.06 | 24.45 | 20.58 | 16.79 |
| 0 to 48 | 17.45 | 25.45 | 22.74 | 17.14 |
| TOTAL RECOVERY | 89.99 | 97.87 | 94.5. | 96.64 |

[a]Data were extracted from Study No. M 182 0176-5, Table I.

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TABLE 2. Percent Recovery of Radioactivity 48 Hours After I.V. or Oral Administration of Imidacloprid (Parts a and b)[a]

| Fraction | Dose Group (% of Recovered Radioactivity) | | | | | |
|---|---|---|---|---|---|---|
| | i.v. 1 mg/kg | p.o. 1 mg/kg | p.o. 1 mg/kg pretreatment | p.o. 20 mg/kg | p.o. 20 mg/kg $CO_2$ test | i.d. 1 mg/kg bile test |
| | | | **Males** | | | |
| $CO_2$ | | | | | 0.033 | |
| BILE | | | | | | 36.57 |
| URINE | 78.71 | 77.77 | 73.80 | 76.99 | 75.77 | 57.52 |
| FECES | 20.73 | 21.71 | 25.47 | 22.33 | 23.61 | 6.78 |
| BODY | 0.56 | 0.52 | 0.72 | 0.68 | 0.58 | [illegible] |
| TOTAL | 100 | 100 | 100 | 100 | 100 | 100 |
| | | | **Females** | | | |
| URINE | 80.21 | 73.68 | 75.43 | 81.89 | | |
| FECES | 19.30 | 25.89 | 23.88 | 17.66 | | |
| BODY | 0.50 | 0.42 | 0.69 | 0.45 | | |
| TOTAL | 100 | 100 [b] | 100 | 100 | | |

[a]Data were extracted from Study Nos. M 182 0176-5, Table II-b and M 181 0175-3, Table 3.

TABLE 3. Percent Recovery of Radioactivity After Oral Administration of [Imidazolidine-4,5-$^{14}$C] Imidacloprid (Part c)[a]

| Fraction | Dose Group (% of Administered Dose) | | | |
|---|---|---|---|---|
| | 1 mg/kg male | 1 mg/kg female | 150 mg/kg male | female |
| URINE | | | | |
| 0 to 4 hours | 41.31 | 39.41 | 9.97 | NR[b] |
| 0 to 8 | 76.32 | 70.10 | 31.29 | NR |
| 0 to 24 | 88.80 | 92.59 | 75.76 | NR |
| 0 to 48 | 89.88 | 93.79 | 90.69 | NR |
| FECES | | | | |
| 0 to 24 hours | 8.09 | 6.08 | 4.40 | NR |
| 0 to 48 | 8.44 | 6.30 | 7.50 | NR |
| URINE + FECES RECOVERY | 98.32 | 100.09 | 98.19 | NR |
| BODY | 1.00 | 0.63 | 1.14 | NR |
| TOTAL RECOVERY | 99.32 | 100.72 | 99.33 | NR |

[a]Data were extracted from Study No. M 31819004, Tables I and III.

[b]Not reported

TABLE 4. Percent Recovery of Radioactivity After Oral Administration of Imidacloprid and WAK 3839 (Part d)[a]

| Fraction | Dose Group (% of Administered Dose) | | | |
|---|---|---|---|---|
| | Imidacloprid 1 mg/kg | WAK 3839 1 mg/kg | Imidacloprid 150 mg/kg | Imidacloprid 20 mg/kg (chronic) |
| URINE | | | | |
| 0 to 4 hours | 39.09 | 51.51 | 5.25 | 8.32 |
| 0 to 8 | 68.47 | 69.62 | 16.26 | 16.91[b] |
| 0 to 24 | 76.71 | 72.38 | 46.75 | 71.36 |
| 0 to 48 | 77.29 | 72.71 | 74.16 | 79.76 |
| FECES | | | | |
| 0 to 24 hours | 20.83 | 14.04 | 6.75 | 5.96 |
| 0 to 48 | 21.37 | 14.26 | 19.73 | 16.62 |
| URINE + FECES RECOVERY | 98.66 | 86.97 | 93.92 | 97.86 |
| BODY | 0.886 | 0.224 | 3.42 | -- |
| TOTAL RECOVERY | 99.55 | 87.19 | 97.31 | 97.86 |

[a]Data were extracted from Study No. M 71810016, Tables I and IV.

[b]Urine was measured after 7 hours instead of 8 hours.

TABLE 5. Percent Recovery of Radioactive Metabolites in Urine 24 Hours After I.V. or Oral Administration of Imidacloprid (Parts a and b)[a]

| Fraction | Dose Group (% of Recovered Radioactivity) | | | |
|---|---|---|---|---|
| | i.v. 1 mg/kg | p.o. 1 mg/kg | p.o. 1 mg/kg pretreatment | p.o. 20 mg/kg |
| | | **Males** | | |
| 6-Chloronicotinic acid | 7.61 | 4.29 | 7.03 | 7.22 |
| Glycine conjugate of 6-S-$CH_2$-nicotinic acid | 2.32 | 2.73 | 4.01 | 2.49 |
| WAK 3583 | 25.90 | 28.11 | 16.58 | 23.61 |
| NTN 35884 | 9.05 | 9.89 | 12.63 | 13.22 |
| WAK 4103[b] | 15.97 | 16.86 | 18.23 | 17.34 |
| Imidacloprid | 13.68 | 11.32 | 10.52 | 8.92 |
| TOTAL IDENTIFIED | 74.53 | 73.20 | 69.00 | 72.80 |
| UNASSIGNED[c] | 4.17 | 4.57 | 4.80 | 4.20 |
| TOTAL RECOVERY | 78.70 | 77.77 | 73.80 | 77.00 |
| | | **Females** | | |
| 6-Chloronicotinic acid | 5.57 | 3.22 | 5.92 | 8.15 |
| Glycine conjugate of 6-S-$CH_2$-nicotinic acid | 5.08 | 5.13 | 5.70 | 3.16 |
| WAK 3583 | 21.66 | 24.11 | 18.89 | 24.23 |
| NTN 35884 | 8.75 | 8.61 | 9.23 | 8.07 |
| WAK 4103[b] | 16.27 | 14.84 | 14.99 | 15.96 |
| Imidacloprid | 14.80 | 11.30 | 12.52 | 15.37 |
| TOTAL IDENTIFIED | 72.13 | 67.21 | 67.25 | 74.94 |
| UNASSIGNED[c] | 8.08 | 6.48 | 8.18 | 6.95 |
| TOTAL RECOVERY | 80.21 | 73.69 | 75.43 | 81.89 |

[a]Data were extracted from Study No. M 182 0176-5, Tables VIII - XV.

[b]Includes 4-OH-Imidacloprid

[c]Includes metabolites 6-hydroxynicotinic acid, 6-methylmercaptanicotinic acid, and the S-Mercapturic acid derivative of nicotinic acid; not quantitated separately due to small amount

TABLE 6. Percent Recovery of Radioactive Metabolites in Feces 24 Hours After I.V. or Oral Administration of Imidacloprid (Parts a and b)[a]

| Fraction | Dose Group (% of Recovered Radioactivity) i.v. 1 mg/kg | p.o. 1 mg/kg | p.o. 1 mg/kg pretreatment | p.o. 20 mg/kg |
|---|---|---|---|---|
| | | **Males** | | |
| Glycine conjugate of 6-S-$CH_3$-nicotinic acid | 1.76 | 1.98 | 2.00 | 2.37 |
| NTN 35884 | 1.32 | 1.13 | 1.20 | 1.71 |
| NTN 33823 | 2.64 | 2.34 | 3.36 | 2.18 |
| Imidacloprid | 1.63 | 2.10 | 1.49 | 0.91 |
| TOTAL IDENTIFIED | 7.35 | 7.55 | 8.05 | 7.17 |
| UNASSIGNED[b] | 6.08 | 6.96 | 6.04 | 6.50 |
| TOTAL RECOVERY | 13.43 | 14.51 | 14.09 | 13.67 |
| | | **Females** | | |
| Glycine conjugate of 6-S-$CH_3$-nicotinic acid | 1.44 | 1.91 | 1.63 | 1.09 |
| NTN 35884 | 0.81 | 1.34 | 1.07 | 0.58 |
| NTN 33823 | 2.40 | 2.43 | 2.96 | 2.21 |
| Imidacloprid | 2.22 | 1.88 | 1.50 | 0.53 |
| TOTAL IDENTIFIED | 6.87 | 7.56 | 7.16 | 4.41 |
| UNASSIGNED[b] | 4.33 | 5.36 | 6.24 | 5.22 |
| TOTAL RECOVERY | 11.20 | 12.92 | 13.40 | 9.63 |

[a]Data were extracted from Study No. M 182 0176-5, Tables VIII - XV.

[b]Includes metabolites 6-hydroxynicotinic acid, 6-methylmercaptonicotinic acid, and the S-Mercapturic acid derivative of nicotinic acid; not quantitated separately due to small amount

TABLE 7. Percent Recovery of Radioactive Metabolites in Urine 24 Hours After Oral Administration of [Imidazolidine-4,5-$^{14}$C] Imidacloprid (Part c)[a]

| Metabolite | Dose Group (% of Recovered Urinary Radioactivity) | | |
|---|---|---|---|
| | 1 mg/kg male | 1 mg/kg female | 150 mg/kg male |
| KHO 0523 | 34.7 | 29.6 | 19.1 |
| NTN 33968 | 8.0 | 15.7 | 18.4 |
| UAK 4283 | 14.7 | 13.7 | 14.6 |
| NTN 3[illegible]64 | 8.4 | 7.7 | 9.1 |
| Imidacloprid | 6.9 | 16.5 | 14.2 |
| TOTALLY IDENTIFIED | 72.7 | 83.2 | 75.4 |
| UNKNOWN[b] | 17.8 | 10.0 | 16.0 |
| TOTAL | 90.6 | 93.2 | 91.4 |

[a]Data were extracted from Study No. M 31819004, Table VI.

[b]The unknown radioactivity represents one single metabolite. This metabolite was "linked to very polar matrix components which prevented further structural elucidation procedures." This metabolite should comprise the imidazolidine moiety only.

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TABLE 8. Percent Recovery of Radioactive Metabolites in Urine 24 Hours After Oral Administration of [Imidazolidine-4,5-$^{14}$C] Imidacloprid and [Methylene-$^{14}$C] Imidacloprid (Part c)[a]

| | Dose Group (% of Recovered Urinary Radioactivity) | | | |
|---|---|---|---|---|
| | [Imidazolidine-4,5-$^{14}$C] 1 mg/kg | | [Methylene-$^{14}$C] 1 mg/kg | |
| Metabolite | Male | Female | Male | Female |
| KNO 0523 | 34.7 | 29.6 | | |
| UAK 3583 | | | 28.1 | 24.1 |
| NTN 33968 | 8.0 | 15.7 | | |
| 6-Cl-Nicot. acid | | | 4.3 | 3.2 |
| 6-S-$CH_3$-Nicot. acid | | | 2.7 | 5.1 |
| UAK 4103 | 14.7 | 13.7 | 16.9 | 14.8 |
| NTN 35884 | 8.4 | 7.7 | 9.9 | 8.6 |
| Imidacloprid | 6.9 | 16.5 | 11.3 | 11.3 |
| TOTALLY IDENTIFIED | 72.7 | 83.2 | 73.2 | 67.1 |

[a]Data were extracted from Study No. M 31819004, Table VII.

TABLE 9. Percent Recovery of Radioactive Metabolites in Urine 24 Hours After Oral Administration of Imidacloprid or WAK 3839 (Part d)[a]

| Metabolite | Dose Group (% of Total Recovered Radioactivity) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Imidacloprid 1 mg/kg | | WAK 3839 1 mg/kg | | Imidacloprid 80 mg/kg (chronic) | | |
| Sampling period (hours) | 0-4 | 4-24 | 0-4 | 4-24 | 0-7 | 7-24 | 24-48 |
| 6-Chloronicotinic acid | 8.68 | 7.10 | | | 12.43 | 7.27 | 2.97 |
| WAK 3583 | 33.84 | 29.38 | | | 31.50 | 21.89 | 0.62 |
| NTN 35884 | 10.08 | 12.38 | | | 18.18 | 17.71 | 15.27 |
| WAK 3839 | 0.33 | 0.76 | 82.16 | 59.40 | 1.36 | 11.41 | 17.24 |
| WAK 4103[b] | 16.43 | 20.74 | | | 6.07 | 14.14 | 11.36 |
| NTN 33823 | | | 6.12 | 9.86 | | | |
| Imidacloprid | 14.03 | 11.08 | | | 20.70 | 10.49 | 5.32 |
| TOTALLY IDENTIFIED | 83.39 | 81.44 | 88.28 | 69.26 | 90.24 | 82.91 | 52.78 |
| UNASSIGNED | 16.61 | 18.57 | 11.71 | 30.74 | 9.85 | 17.09 | 47.23 |
| TOTAL | 100.00 | 100.01 | 99.99 | 99.98 | 100.09 | 100.00 | 100.01 |

[a]Data were extracted from Study No. M 71810016, Table VI.

[b]Includes 4-OH-Imidacloprid

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TABLE 10. Percent Recovery of Radioactive Metabolites in Urine and Feces 24 Hours After Oral Administration of Imidacloprid or WAK 3839 (Part d)[a]

| Metabolite | Dose Group (% of Total Recovered Radioactivity) Imidacloprid 1 mg/kg | WAK 3839 1 mg/kg |
|---|---|---|
| 6-Chloronicotinic acid | 6.51 | |
| WAK 3583 | 24.61 | |
| NTN 35884 | 9.51 | |
| WAK 3839 | 0.42 | 62.76 |
| WAK 4103[b] | 14.29 | |
| NTN 33823 | | 5.98 |
| Imidacloprid | 10.60 | |
| TOTALLY IDENTIFIED | 65.58 | 68.74 |
| UNASSIGNED | 32.41 | 14.28 |
| TOTAL | 97.99 | 83.02 |

[a]Data were extracted from Study No. M 71810016, Table X.

[b]Includes 4-OH-Imidacloprid

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Toxicology Branch I, (IRS)/HED (H7509C)

03-24-93

8/27/93

DATA EVALUATION REPORT

I. SUMMARY

Study Type: (84-2) Mutagenicity

MRID No.: 422563-67
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.:D180299/D179336

Chemical: NTN 37571 (. metabolite of NTN 33893)

Synonymns: NTN 37571

Sponsor: MOBAY, Kansas City

Testing Facility: Nikon Tokushu Noyaku Seizo KK, Tokyo (Japan)

Title of Report: NTN 37571: Micronucleus Test on the Mice After I.P. Treatment Pilot Study

Author: M. Usami

Study No.: 88S032 (Report No. RS 88041/100679)

Report Issued: November 29, 1988

TB Conclusions: Test article was severely toxic at i.p. 100 mg/kg (2/5 animals died), but not at 75 mg/kg or below. No clastogenic effect (increased micronuclei) or cytotoxicity was found at doses up to 80 mg/kg.

TB-I Evaluation: [Not evaluated; pilot only]

II. DETAILED REVIEW:

A. Test Material: NTN 37571 (Kyowa Hakko)

Description: Powder
Batch (Lot): TX 19088
Purity (%): 96.5
Solvent/carrier/diluent: Dimethylsulfoxide/olive oil

B. Test Organism: Rodent

Species: Mouse
Strain: BD-$F_1$
Age: 8 weeks
Weight: Males (only)
Source: Charles River Japan, Tokyo

C. Study Design (Protocol):

This pilot study was designed to determine the dosages of the test article to be administered to mice in a micronucleus assay.

D. Procedures/Methods of Analysis:

Following preliminary toxicity testing, test article was administered once intraperitoneally (i.p.) to mice (5/group) at dosages of 20, 40 and 80 mg/kg and sacrificed 30 hours later. A final group received mitomycin-C (MMC, 4 mg/kg), to serve as positive control.

At sacrifice femoral bone marrow was prepared by conventional smear technique on standard glass microscope slides, and 500 polychromatic erythrocytes (PCE) per animal scored for micronuclei. Ratios of PCE to NCE were also determined.

E. Results: In the preliminary study, two of five mice died shortly after the ip administration of 100 mg/kg, but no deaths were recorded below that level (Report Table 1). In the main study, ip doses up to 80 mg/kg had no effect n the incidence of micronuclei or PCE/NCE ratios (i.e., no cytotoxicity was demonstrated).

F. TB-I Evaluation: Cannot be evaluated, hence UNACCEPTABLE since: (i) it was a pilot study; (ii) no toxicity was demonstrated at the HDT, and (iii) the results were at variance with another parenteral study, T-0032852 (MRID 422563-66).

Attachments (Data Tables)

NTN37571.IM/lca

Toxicology Review # 010537, 9/3/93

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Pages 107 through 108 are not included.

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Reviewed by: Irving Mauer, Ph.D., Geneticist, 03-29-93
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C) 3/29/93

DATA EVALUATION REPORT

I. SUMMARY

Study Type: (84-2) Mutagenicity - Chromosome aberrations in vivo (mouse MT)

MRID No.: 422563-68
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.:D180299/D179336

Chemical: WAK 3839 (metabolite of NTN 33893)

Sponsor: MOBAY, Kansas City

Testing Facility: Bayer AG, Wuppertal (FRG)

Title of Report: WAK 3839 or NTN 37571: Micronucleus Test on the Mouse After Oral Application

Author: B. A. Herbold

Study No.: T1032853 (Report No. 18406/100663)

Report Issued: October 3, 1989

TB Conclusions: Reportedly negative for the induction of micronuclei in bone marrow cells from mice treated once orally at 100 mg/kg test article, a cytotoxic dose.

TB-I Evaluation: Acceptable

II. DETAILED REVIEW:

A. Test Material: WAK 3839 (Bayer AG)

Description: Yellow-greenish powder
Batch (Lot): WAK 3839/C-E
Purity (%): 98.9
Solvent/carrier/diluent: 0.5% Aqueous Cremophor Emulsion (CMC)

B. Test Organism: Rodent

Species: Mouse
Strain: Bor:NMRI (SPF Han)
Age: 8-12 weeks
Weights: Males/females: 28-40 g
Source: F. Winkelman, Borchen (FRG)

C. Study Design (Protocol):

This study was designed to assess the clastogenic potential of the test article when administered in vivo to mice, and assaying for micronuclei in bone marrow PCE, according to established (published) procedures and FIFRA Test Guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following dose-selection testing, groups of mice (5 male: 5 female/group) were given test article once by oral gavage and sacrificed 24, 48 or 72 hours later. Concurrently, other groups sacrificed only at 24 hours after treatment were given Cremophor only, or the mutagen cyclophosphamide (CP), to serve as, respectively, vehicle and positive controls.

At sacrifice, femoral bone marrow was smeared onto glass microscope slide, stained with modified H&E, and prepared for microscopy. Codes slides were scanned under oil immersion, and 1000 polychromatic erythrocytes (PCE) scored for the presence of micronuclei (m-PCE). In addition the ratio of PCE to normochromatic erythrocytes (NCE) was determined, as an indirect measure of cytotoxicity.

Wilcoxon's (non-parametric) Rank Sum Test was employed to ascertain significance of any differences between control

and test group values, with alpha set at 5%. Conventionally acceptable criteria for assay acceptance and evaluation of (clastogenic) responses were also presented.

E. Results: In the preliminary pilot study, four of five animals given 250 mg/kg WAK 3839 died but only 1 of 10 given 100 mg/kg. Dose-related clinical toxicity at both doses included: Apathy, staggered gait, and rales. Hence 100 mg/kg was selected as the HDT for the main study.

In the main study, all animals manifested some degree of the same clinically adverse signs, but there were no deaths. In none of the test animals (Report Tables 1-5), nor summary means calculated for each time group (Report Table 6, attached here) was there any evidence of cytotoxicity, nor any significant changes in m-PCE. By contrast, CP-treated animals responded with clearly significant increases in micronuclei, again in the absence of any cytotoxicity.

Hence, the investigator concluded that WAK 3839 was not clastogenic in this micronucleus test.

F. TB-I Evaluation: Acceptable

Attachments (Data Tables)

WAK3839.IM/lca

Toxicology Review # 010537 9/3/93

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Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

1-5-93

5/29/92

DATA EVALUATION RECORD

010537

MRID NUMBER No.: 422563-69
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU (NTN 33893 tech)
Tox Chem. No.: 497E
Project No.: D180299/D179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---Chromosome aberrations in vivo (mouse MT)

CHEMICAL: WAK 3839 (metabolite of NTN 33893)

SYNONYMNS: NTN 37571

SPONSOR: Mobay, KC

TESTING FACILITY: Nihon Tokushu Noyaku Seizo KK, Tokyo (Japan)

TITLE OF REPORT: Micronucleus Test on the Mice After Oral Treatment. Pilot Study

AUTHOR: M. Usami

STUDY NUMBER: 88S031 (Report # RS 88040/100680)

DATE ISSUED: November 29, 1988

CONCLUSIONS: 160 Mg/kg was selected as the HDT for the projected cytogenetic study in mice.

TB-I EVALUATION: (Not graded; pilot study only)

## II. DETAILED REVIEW

A. TEST MATERIAL: NTN 37571

| | |
|---|---|
| Description: | [Not stated] |
| Batches (Lots): | TX 19088 |
| Purity (%): | 96.4 |
| Solvent/carrier/diluent: | Dimethylsulfoxide (DMSO)/polyethylene glycol 400 (PEG) |

B. TEST ORGANISM: Rodent

| | |
|---|---|
| Species: | Mouse |
| Strain: | [Not stated] |
| Age: | 9 weeks |
| Weights - males: | [Not stated] |
| females: | [Not stated] |
| Source: | [Not stated] |

C. STUDY DESIGN (PROTOCOL): This pilot study was designed to select doses of the test article to be administered orally to mice in a projected chromosome aberration study for the detection of micronuclei in bone marrow PCE.

D. PROCEDURES/METHODS OF ANALYSIS: Following preliminary dose-selection testing, groups of mice (5/group) were administered test article at single oral doses of 40, 80 and 160 mg/kg and sacrificed 30 hours later. In addition to vehicle (PEG) controls, a further group of animals was given mitomycin: (MMC, 4 mg/kg) by intraperitoneal injection.

At sacrifice, femoral bone marrow was smeared onto glass slides, fixed in methanol, and stained with Giemsa.

The incidence (0/00) of micronucleated polychromatic erythrocytes (m-PCE) among 500 cells, as well as the ratio of PCE to normochromatic erythrocytes (NCE), were determined for each animal (the latter as an indirect measure of cytotoxicity).

E. RESULTS: In preliminary testing, death occurred in 1 of 5 mice given 200 mg/kg, 3 of 5 at 300 mg/kg, and all 5 at 400 mg/kg, but none at 160 mg/kg or below (Report Table 1). Therefore, 160 mg/kg was selected as the HDT for the main study.

In the main study, one high-dose mouse died; autopsy revealed renal hydronephrosis, conceivably congenital. There were no differences from vehicle control values in incidence of m-PEC of m-PEC among test animals, and no evidence of cytotoxicity. By contrast, the MMC - treated group showed clear cytogenetic effect, by a marked increase in m-PCE over background. (Report Table 2.)

F. TB EVALUATION: [Supplementary data only, as a pilot study hence no graded].

ATTACHMENT: (Data Tables)

Disk #7/mb/422563.69

010537

Reviewed by: Irving Mauer, Ph.D., Geneticist 03-12-93
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief 8/27/93
Toxicology Branch-I, HED (H7509C)

DATA EVALUATION RECORD

MRID NUMBER No.: 422563-63
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem. No.: 497E
Project No.: D180299/179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---Ames Test

CHEMICAL: WAK 3839 (metabolite of NTN 33893, imidaclopid)

SPONSOR: Mobay, KC

TESTING FACILITY: Hino Institute, Tokyo (Japan)

TITLE OF REPORT: WAK 3839: Reverse Mutation Assay (Salmonella typhimurium and Escherichia coli)

AUTHOR: M. Watanabe

STUDY NUMBER: 90A015 (Report #RA90035/100668)

DATE ISSUED: November 26, 1990

CONCLUSIONS: Negative for reverse gene mutation in two bacterial species exposed up to limit dosages, (5000 ug/plate), with or without activation

TB-I EVALUATION: ACCEPTABLE

II. DETAILED REVIEW

A. Test Material: WAK 3839

Description: Yellow crystals
Batches (Lots): TX 020390
Purity (%): 98.3
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Bacterial cultures

Species: Salmonella typhimurium; Escherichia coli
Strains: TA 98, TA 100, TA 1535, TA 1537 (all his-); WP2/uvr A (tryp-)
Source: Institute of Environmental Toxicology, Tokyo (Japan)

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the mutagenic potential of the test article when administered in vitro to bacterial cultures of Salmonella typhimurium and Escherichia coli, and assaying for reverse mutations, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following preliminary cytotoxicity testing, triplicate cultures of the above indicated test organisms were exposed to DMSO (solvent control) or to each of five graded concentrations of the test article, in the absence as well as presence of a mammalian activation system consisting of the microsomal (post-mitochondrial, S9) fraction of livers from rats pretreated with the hepatic enzyme stimulaters phenobarbital and 5,6-benzoflavone, plus NADP (H)-generating cofactors (S9 mix). After incubation for 48 hr, revertent colonies (his+; tryp+) were counted. Mutagens appropriate to each strain served as positive controls. The entire assay was repeated once. A test substance is considered positive in this lab if the mean incidence of revertent counts in test cultures is increased two-fold or more over the solvent control values and this increase is dose-related.

E. **Results**: No significant increases in revertent counts observed in any test culture in two trials at concentrations up to 5000 ug per plate (Report Tables 1, 2, attached here). In contrast, all positive control cultures exposed to the strain-specific mutagens responded with marked increases in revertents.

Therefore the investigator concluded that WAK 3839 was not mutagenic in gene mutation assays with two species of bacteria.

F. **TB Evaluation**: Acceptable

Attachment (Data Tables)

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Toxicology Review # 010537 9/3/93

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010537

Reviewed by: Irving Mauer, Ph.D., Geneticist,
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C)

3-17-93

8/24/93

DATA EVALUATION REPORT

I. SUMMARY

Study Type: (84-2) Mutagenicity

MRID No.: 422563-64
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-LRU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.: D180299/D179336

Chemical: WAK3839 (Metabolite of NTN 33893, imidacloprid)

Sponsor: MOBAY, Kansas City

Testing Facility: Bayer AG, Wuppertal (FRG)

Title of Report: Mutagenicity Study for the Detection of Induced Foreward Mutations in the V79-HGPRT Assay in vitro

Author: H. Lehn

Study No.: T8030618 (Report No. 18281/100662)

Report Issued: August 15, 1989

TB Conclusions: Negative for inducing foreward mutations at the HGPRT locus of V79 cells exposed, with/without activation, to the solubility limit (2000 ug/ml).

TB-I Evaluation: Acceptable

II. DETAILED REVIEW:

A. Test Material: WAK 3839 (Bayer AG)

Description: Yellow-green powder
Batch (Lot): WAK 3839/C-E
Purity (%): 98.9
Solvent/carrier/diluent: Tissue culture medium

B. Test Organism: Established mammalian cell strain

Species: Chinese hamster (lung)
Strain: V79
Source: Dr. E. Müller, CCR, Darmstadt (FRG)

C. Study Design (Protocol):

This study was designed to assess the mutagenic potential of the test article when administered in vitro to cultures of Chinese hamster lung cells (V79), and assaying for foreward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus, according to established (published) procedures and FIFRA Test Guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following preliminary toxicity testing ( up to the limit of solubility in tc-medium, 2000 ug/ml), duplicate cultures of V79 cells were exposed for 5 hrs to test article, in the absence as well as presence of a mammalian metabolic activation system, consisting of the post-mitochondrial (S9) fraction of livers from rats pretreated with the PCB enzyme inducer, Aroclor 1254 (purchased from Cytotest Cell Research, Darmstadt), plus freshly prepared NADP(H)-generating co-factors. Following such treatment, cell microlayer cultures were washed and incubated for 5-7 days (to allow expression of mutant colonies, and determine growth/survival), then exposed for a further 7 days to 6-thioguanine (TG) which selects for mutants ($HGPRT^{-/-}$), and against normal cells ($HGPRT^{+/-}$). The mutagens, ethylmethansulfonate (EMS) and dimethylbenzanthracene (DMBA) served as positive controls for, respectively, non-activation and activation series. The entire assay was repeated once.

The following parameters were determined:

$$RS^{1}\ (RCE) = \frac{\text{Av. no. colonies per treated culture}}{\text{Av. no. colonies per vehicle control}} \times 100$$

$$RPG = \frac{\text{Treated culture popu. incr. during expression}}{\text{Vehicle control popu. incr. during expression}} \times 100$$

$$ACE = \frac{\text{Av. No. viable colonies per dish}}{200} \times 100$$

$$MF = \frac{\text{Total no. mutant (TG-resistant) colonies}}{10^{6} \text{ clonable cells}}$$

Conventionally accepted criteria for assessing mutagenic responses in analyzable assays meeting the laboratory's strict criteria were presented. Statistical treatment of data included the Poisson heterogeneity test to determine significant increases in MF, with a type I (alpha) error rate adjusted to account for the multiplicity of tests.

E. Results: In preliminary dose-selection screening, RS was close to 50% at levels close to the solubility limit (1500-2000 ug/ml) in non-activation cultures, but was only moderately affected (80%) in the presence of S9. Hence, five concentrations ranging from 500 to 2000 ug/ml were selected for the first mutagenicity trial, a dosage scheme modified slightly for the second trial.

Only two of the four mutagenicity trials initiated under non-activation conditions were acceptable for analysis. In both of these, dose-related cytotoxicity was observed up to 2000 ug/ml, the limit of solubility, but no consistent increases in MF (Report Tables 3 and 4 attached here). Only two of the five trials initiated with S9 could be analyzed, and these also did not yield any significant increases in MF (Report Tables 5 and 6, attached here). By contrast, both mutagens incurred marked increases in MF.

Therefore the author concluded that WAK 3839 was not mutagenic in V79 cells.

F. TB-I Evaluation: Acceptable

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[1] RS, relative survival compared to vehicle control (%)
RCE, relative cloning efficiency (%)
RPG, relative population growth (%)
ACE, absolute cloning efficiency (%)
MF, mutant frequency

Toxicology Review # 010537 9/3/93

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Reviewed by: Irving Mauer, Ph.D., Geneticist,
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C)

03-23-93
6/29/93

010537

DATA EVALUATION REPORT

I. SUMMARY

Study Type: Gene Mutation in mammalian cells in vitro (CHO/HGPRT)

MRID No.: 422563-65
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.:D180299/D179336

Chemical: WAK 3839 (metabolite of NTN 33893, imidacloprid)

Sponsor: MOBAY, Kansas City

Testing Facility: Bayer AG, Wuppertal (FRG)

Title of Report: Mutagenicity Study for the Detection of Induced Foreward Mutations in the CHO-HGPRT Assay in vitro

Author: H. Lehn

Study No.: T7030167 (Report No. 17757/100661)

Report Issued: February 22, 1989

TB Conclusions: Negative for inducing foreward mutations at the HGPRT locus of CHO cells exposed, with/without activation, up to cytotoxic and/or precipitating dose, 2000 ug/ml).

TB-I Evaluation: Acceptable

II. DETAILED REVIEW:

A. Test Material: WAK 3839 (Bayer AG)

Description: Green powder
Batch (Lot): WAK 3839/C-E
Purity (%): 94.3
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. Test Organism: Established mammalian cell strain

Species: Chinese hamster (ovary), CHO
Strain: $K_1$-$BH_4$ ($HGPRT^{+/-}$)
Source: Dr. A.W. Hsie, ORNL (TN)

C. Study Design (Protocol):

This study was designed to assess the mutagenic potential of the test article when administered in vitro to cultures of CHO cells, and assaying for mutations at the HGPRT locus, according to established (published) procedures and FIFRA Test Guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following preliminary dose-selection testing, duplicate cultures of CHO cells were exposed for 5 hrs to DMSO alone (solvent control), or to graded concentrations of test article, both in the absence and presence of mammalian metabolic activation consisting of the post-mitochondrial fraction (S9) of livers from rats pretreated with the enzyme inducer, Aroclor 1254 (purchased from Litton Bionetics), plus NADP(H)-generating co-factors. After removal of treatment medium, cells were sub-cultured for 7 days (to express mutants), then exposed for a further week to 6-thioguanine (TG), which selects for mutants. The mutagens, ethylmethansulfonate (EMS), and dimethylbenzanthracene (DMBA) served as positive controls. Two complete independent assays were performed.

The following parameters were determined:

$$RS^1 (RCE) = \frac{\text{Av. no. colonies per treated culture}}{\text{Av. no. colonies per vehicle control}} \times 100$$

$$RPG = \frac{\text{Treated culture popn. incr. during expression}}{\text{Vehicle control popn. incr. during expression}} \times 100$$

$$ACE = \frac{\text{Av. No. viable colonies per dish}}{200} \times 100$$

$$MF = \frac{\text{Total no. mutant (TG-resistant) colonies}}{10^5 \text{ clonable cells}}$$

Conventionally accepted criteria for assay acceptance and mutant response were followed by this lab. The Poisson heterogeneity test for significant differences in MF was run, and Type I (alpha) error rate adjusted to account for the multiplicity of tests.

E. Results: In preliminary toxicity screening, the test article was lethal at doses of 100 ug/ml and above in non-activation cultures (RS was near-normal, 82% at 50 ug/ml), but non toxic up to the HDT, 800 ug/ml, in the presence of S9 (Report Tables 1, 2.)

In two of three acceptable mutation assays with activation, WAK 3839 was tested up to 2000 ug/ml, and produced dose-related decreases in RS, but no increase in mutant colonies (Report Tables 3 and 4, attached here). In the presence of S9, doses up to the limit of solubility (2000 ug/ml) produced minimal cytotoxicity, but again no induced mutation (Report Tables 5 and 6, attached). By contrast, both positive controls responded with highly significant increases in MF.

The investigators concluded that WAK 3839 was deemed non-mutagenic in this assay.

F. TB-I Evaluation: Acceptable

Attachments (Data Tables)

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[1] RS, relative survival compared to vehicle control (%)
RCE, relative cloning efficiency (%)
RPG, relative population growth (%)
ACE, absolute cloning efficiency (%)
MF, mutant frequency

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Toxicology Review # 010537 9/3/93

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010537

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

01-22-93

8/27/93

DATA EVALUATION RECORD

MRID NUMBER No.: 422563-70
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU (NTN 33893 tech)
Tox Chem. No.: 497E
Project No.: D180229/D179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---Chromosomal aberrations in vitro (V79)

CHEMICAL: WAK 3839 (metabolite of NTN 33893)

SYNONYMNS: NTN 37571

SPONSOR: Mobay (Bayer), KC

TESTING FACILITY: Cytotest Cell Research GMBH, Robdorf (FRG)

TITLE OF REPORT: Chromosome Aberrations in Chinese Hamster V79 Cells in vitro with WAK 3839

AUTHOR(S): A. Heidemann

STUDY NUMBER: T1032772/151200 (Report No. R4849/100666)

DATE ISSUED: September 27, 1989

CONCLUSIONS: Negative for inducing chromosome aberrations in Chinese hamster lung (V79) cells, exposed in vitro up to precipitating doses (1,300 ug/ml), with/without activation.

TB-I EVALUATION: ACCEPTABLE.

## II. DETAILED REVIEW

A. TEST MATERIAL: WAK 3839 (Bayer AG)

Description: White-yellowish powder
Batches (Lots): WAK 3839/C-E
Purity (%): 98.8
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. TEST ORGANISM: Established mammalian cell strain

Species: Chinese hamster (lung)
Strain: V79
Source: LMP, Darmstadt (FRG)

C. STUDY DESIGN (PROTOCOL): This study was designed to
- assess the clastogenic potential of the test article
- when administered in vitro to V79 cells, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following preliminary cytotoxicity testing, duplicate cultures of CHL-V79 cells were exposed for 4 hours to graded concentrations of test article (or control substances), both in the absence and presence of a mammalian metabolic activation system, consisting of the microsomal fraction (S9) of livers from male Wistar rats pretreated with the PCB enzyme inducer, Aroclor 1254, plus NADP(H)-generating co-factors (=S9 Mix). Solvent (DMSO), and ethylmethane sulfonate (EMS) or cyclophosphamide (CP), served as negative and positive controls the latter for, respectively, non-activation and activation test series.

Test cultures were harvested 7, 18 and 28 hours following removal of test article, with the mitotic inhibitor, Colcemid, added 2 hours prior to harvest. Cells were expanded (in hypotonic saline), fixed in methanol acetic acid, and finally stained with Giemsa on standard glass microscope slides.

Coded slides were scanned under oil immersion and morphologically normal (2N=22±1) metaphases scored for the conventional array of structural chromosome aberrations (primary cytogenetic damage), as well as for mitotic index (as a measure of cytotoxicity).

This lab considers a test substance positive (in an acceptable assay only) if it induces either a significant dose-related increase in the number of aberrations, or a significant and reproducible positive response for at least one of the test points. A test article producing neither a significant dose-related increase in the number of structural chromosomal aberrations nor a significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system. This is confirmed by means of the nonparametric Mann-Whitney test.

E. RESULTS: In preliminary dose selection tests, WAK 3839 precipitated at concentrations of 1,300 µg/ml, a toxic dose in the absence of activation (Report Tables 1 to 3). However, in contrast to the significant positive results in response to the referenced mutagens, at no dose in replicate experiments with/without activation, did the test article induce increased structural chromosomal aberrations above solvent control values (Report Tables 4 through 23, summarized in Tables 24, 25 and 26, attached here). The authors concluded that the test article was not clastogenic in V79 cells up to the limits of solibility in tissue culture medium.

F. TB EVALUATION ACCEPTABLE



ATTACHMENT: (Data Tables)

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8/27/93

Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

# DATA EVALUATION RECORD

MRID NUMBER No.:422563-71
PC No.:129099
RD Record No.:S419490
EPA ID No.:003125-URU (NTN 33893 Tech)

Tox Chem. No.:497E
Project No.:D180229/D179336

## I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity --Chromosome aberrations in vitro (CHO cells)

CHEMICAL: NTN 37571 (metabolic of NTN 33893)

SYNONYMNS: WAK 3839

SPONSOR: Mobay, KC

TESTING FACILITY: Nihon Tohushu Noyaku Seizo KK (Japan)

TITLE OF REPORT: In Vitro Cytogenetic Assay Measuring Chromosome Aberrations in CHO-K1 Cells

AUTHOR: M. Usami

STUDY NUMBER: 88PO16 (Report #RP880088/100678)

DATE ISSUED: November 5, 1988

CONCLUSIONS: Negative for inducing chromosome aberrations in Chinese hamster ovary (CHO) cells exposed up to cytotoxic levels (1000 µg/ml), with or without mammalian metabolic activation.

TB-I EVALUATION: ACCEPTABLE

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## II. DETAILED REVIEW

A. TEST MATERIAL: NTN 37571 (Wako Chemical)

Description: White-yellow powder
Batches (Lots): WAK 3839
Purity (%): 98.8
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. TEST ORGANISM: Established mammalian cell strain

Species: Chinese hamster (ovary)
Strain: CHO-K1
Source: Dainippon Pharmaceutical

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the clastogenic potential of the test article when administered in vitro to Chinese hamster ovary (CHO) cells, according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: After preliminary dose-selection testing, duplicate cultures of CHO cells were exposed to graded concentrations of test article in the absence of activation for 24 or 48 hours, but only for 4 hour (plus 20 hour post-treatment incubation in fresh tissue culture medium) in the presence of a mammalian metabolic activation system (rat liver S9, purchased from Kikkomen) plus NADP(H)-generating co-factors. The mammalian metabolic activation system (rat liver S9, purchased from Kikkomen) plus NADP(H)-generating co-factors. The mutagens, MNNG (methyl-nitro-nitrosoguanidine) and DMN (dimethylnitrosamine) served as positive controls for, respectively, the non-activation and activation series.

E. RESULTS: In the preliminary dose-selection tests, the test article was moderately toxic at 1000 ug/ml (relative growth rate = 50%) without activation but only minimally (84% RG) +S9 (Tables 1, 3). However, in contrast to the statistically increased frequency of aberrations produced by the positive control substances, no increased clastogenesis over control values was found with the test article. (Tables 2 and 4, attached here)

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F. TB EVALUATION: Acceptable

ATTACHMENTS (Data Tables)

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Pages 155 through 156 are not included in this copy.

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_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

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Reviewed by: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I, HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I, HED (H7509C)

01-12-93

DATA EVALUATION RECORD

MRID NUMBER No.: 422563-72
PC No.: 129099
RD Record No.: S419490
EPA ID No.: 003125-URU (NTN 33893 tech)
Tox Chem. No.: 475E
Project No.: D180229/D179336

I. SUMMARY

STUDY TYPE: (84-2) Mutagenicity---DNA damage/repair in vitro (HPC/UDS)

CHEMICAL: WAK 3839 (metabolite of NTN 33893)

SYNONYMNS: NTN 37571

SPONSOR: Mobay, KC

TESTING FACILITY: Cytotest Cell Research, Darmstadt (FRG)

TITLE OF REPORT: Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro with WAK 3839.

AUTHOR: R. Fautz

STUDY NUMBER: T4030074 (Report # R4746/100665)

DATE ISSUED: April 24, 1989

CONCLUSIONS: Negative for induced DNA damage repair in rat hepatocyte cultures, as represented by increased grain counts indicating unscheduled DNA synthesis, treated up to cytotoxic doses (1333 ug/ml)

TB-I EVALUATION: ACCEPTABLE

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II. DETAILED REVIEW

A. TEST MATERIAL: WAK 3839 (Wako Chemical)

Description: White-yellowish powder
Batches (Lots): WAK 3839/C-E
Purity (%): 98.9
Solvent/carrier/diluent: Dimethylsulfoxide (DMSO)

B. TEST ORGANISM: Primary hepatocyte cultures

Species: Rat
Strain: Wistar CF HB
Age: 8-12 weeks
Weights - males: 180-240 g
Source: SAVO Ivanovas (Kisslegg FRG)

C. STUDY DESIGN (PROTOCOL): This study was designed to assess the genotoxic potential of the test article when administered in vitro to cultures of primary rat hepatocytes, and measuring unscheduled DNA synthesis (as increased nuclear silver grain count), according to established (published) procedures and FIFRA Test Guidelines.

A Statement of Quality Assurance measures (inspections/audits) was provided.

A Statement of adherence to Good Laboratory Practice (GLP) was provided.

D. PROCEDURES/METHODS OF ANALYSIS: Following cytotoxicity testing, hepatocytes isolated from male rats were exposed as coverslip cultures for 18 hours to graded concentrations of test article together with tritiated thymidine ($^3$H-TdR, 5uCi/ml; sp. act., 20 Ci/mmol). After this treatment, coverslip cultures were expanded in 1% sodium citrate, fixed in Carnoy's and mounted on standard glass microscope slides. The slides were then dipped (under safelight) in photographic emulsion (Ilford K-2), dried and stored under refrigeration in light-tight boxes. After seven days storage, slides were developed in standard photographic solutions, stained with aceto-orcein, and covered for microscopic examination.

Under oil immersion, at least 100 cells per dose level were scored for photographic silver grains over hepatocyte nuclei, and net nuclear grain counts (NNGC) determined (as a measure of unscheduled DNA synthetic repair of damage). The

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mutagen 2-acetoaminofluorene (AAF) served as positive control. The assay was repeated twice (three trials in toto).

E. RESULTS: In preliminary testing, doses of 444 mg/ml and above were toxic, and 1333 mg/ml and above precipitated out. Hence in the repeat experiments the following doses were scored:

| | |
|---|---|
| EXP-I: | 8 concentrations from 0.04 to 133.33 ug/ml |
| EXP II: | 10 concentrations from 0.04 thru 1333.33 ug/ml |
| EXP III: | 5 concentrations from 13.33 to 1333.33 ug/ml |

Although isolated increased grain counts were encountered in the first two trials, no reproducible dose-dependent increases in NNGC were found (see Data Tables, attached here). The reference mutagen, AAF, produced significantly increased UDS (as measured by grain counts).

F. TB EVALUATION: ACCEPTABLE.

ATTACHMENT (Data Tables

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# TABLES OF RESULTS

## EXPERIMENT I

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Reviewed by: Irving Mauer, Ph.D., Geneticist,
Toxicology Branch I, (IRS)/HED (H7509C)
Secondary Reviewer: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch I, (IRS)/HED (H7509C)

3-24-93

8/27/93

# DATA EVALUATION REPORT

## I. SUMMARY

Study Type: (84-2) Mutagenicity - Chromosome aberrations in vivo (Mouse MT)

MRID No.: 422563-66
PC No.: 129099
RD Record No.: S-419490
EPA ID No.: 003125-URU
(NTN 33893 Tech)
Tox Chem No.: 497E
Project No.:D180299/D179336

Chemical: WAK 3839 (metabolite of NTN 33893)

Synonymns: NTN 37571

Sponsor: MOBAY (Miles), Kansas City

Testing Facility: Bayer AG, Wuppertal (FRG)

Title of Report: WAK 3839 or NTN 37571: Micronucleus Test on the Mouse After Intraperitoneal Injection

Author: B. A. Herbold

Study No.: T0032852 (Report No. 18407/100664)

Report Issued: October 3, 1989

TB Conclusions: Negative for inducing micronuclei in PCE of mice treated parenterally at the MTD (50 mg/kg)

TB-I Evaluation: Acceptable

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II. DETAILED REVIEW:

A. Test Material: WAK 3839 (Bayer AG)

Description: Yellow-greenish powder
Batch (Lot): WAK 3839/C-E
Purity (%): 98.9
Solvent/carrier/diluent: 0.5% Aqueous Cremophor Emulsion (CMC)

B. Test Organism: Rodent

Species: Mouse
Strain: Bor:NMRI (SPF Han)
Age: 8-12 weeks
Weights: Males/females: 31-41 g
Source: F. Winkelman, Borchen (FRG)

C. Study Design (Protocol):

This study was designed to assess the clastogenic potential of the test article when administered i.p. to mice, and evaluating the induction of micronuclei in PCE, according to established (published) procedures and FIFRA Test Guidelines.

Statements of both Quality Assurance measures (inspections/audits) as well as of adherence to Good Laboratory Practice (GLP) were provided.

D. Procedures/Methods of Analysis:

Following dose-selection testing, groups of mice (5 male: 5 female/group) were injected once i.p. with 50 mg/kg test article, and sacrificed 24, 48 or 72 hours later. Two further groups were given Cremophor or cyclophosphamide (CP) to serve as negative and positive controls, respectively.

At sacrifice, femoral bone marrow was prepared by standard cytological procedures as smears on microscope slides, stained with H and E, and sealed under coverslips. One thousand polychromatic erythrocytes (PCE) per animal on coded slides were scored for the presence of micronuclei (m-PCE), as well as determining the ratio of PCE to normochromatic erythrocytes (indirect evidence of cytotoxicity).

Wilcoxon's (non-parametric) Rank Sum Test was used to analyze the resulting data, with alpha set at 5%.

Conventionally acceptable criteria for assay acceptance as well as for responses were provided in the Final Report.

E. Results: In the preliminary pilot (dose-selection) study, all animals given 100 mg/kg i.p. died, but only 1 of 10 at 50 mg/kg. Dose-related clinical toxicity was noted, starting at 25 mg/kg, as follows: Apathy, staggering gait, rales. Hence 50 mg/kg was selected at the MTD for the main study.

The same syndrome of adverse clinical signs was evident in the main study at 50 mg/kg in all groups, but no mortalities. Whereas the ratio of PCE to NCE was slightly altered (indicating that the test article reached the target tissue to produce toxicity) no statistically significant or biologically relevant differences from negative control values were recorded in any test group (Report tables 1 through 5, attached here). By contrast, the positive control, CP, manifested clearly clastogenic effects.

Hence, the author concluded that WAK 3539 was not clastogenic in inducing micronuclei in bone marrow cells of mice treated parenterally at a clearly toxic dose.

F. TB-I Evaluation: Acceptable

Attachments (Data Tables)

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