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

#497E

011070

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: I.D. Nos.: 007501-RLL, 4F04337, 007501-RLO. Imidacloprid. Evaluation of Toxicity Data Submitted and Identification of Outstanding Toxicology Data Requirements

Tox. Chem. No.	497E
PC Code No.	125099 (125091)
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I. CONCLUSIONS

The existing database supports the following uses for Imidocloprid:

Chemical I.D.	Submission	Formulation	Application
007501-RLL	Registration	GAUCHO 480 Flowable	Cotton
4F04337	Tolerance Petition	Imidacloprid	Cotton
007501-RLL	Registration	GAUCHO 480 Flowable	Wheat, barley, sorghum & sugar beets
007501-RLO	Registration	GAUCHO 75 ST	Sugar beets

II. ACTION REQUESTED

TB-1 received for evaluation the studies required to fulfill data requirements for registration of NTN 33893 Technical and Formulations (40% and 75%) for food use. These data were submitted by Bayer AG, Miles, Inc., and Gustafson, Inc. In addition, TB1 was asked to evaluate the database for several other uses of Imidacloprid.

III. RESULTS/DISCUSSION

The requirements (CFR 158.135) for Food Use for the Technical are listed in the following table.

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	-

Test		Technical		Formulations§	
		Required	Satisfied	Required	Satisfied
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	N	-	N	-
85-2	Dermal Penetration	N	-	N	-
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	-

Y yes
N no
§ GAUCHO 480 Flowable and GAUCHO 75 ST 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.

Data Gaps No outstanding data gaps have been identified for these formulations.

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 75% and 40% Formulations, respectively.

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

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

TABLE 2B. SUMMARY OF ACUTE TOXICITY OF NTN 33893 75% FLOWABLE FORMULATION

CITATION	RESULTS	CATEGORY
Acute Oral/Rat Mobay 91-012-JJ Aug. 27, 1991 MRID 422563-12	LD_{50}: Males: 2591 mg/kg, Slope = 2.3 Females: 1858 mg/kg, Slope 5.4 NOEL: <1063 mg/kg Study is Acceptable Toxic Signs: Clinical: tremors, increased reactivity, decreased activity, eyes partially shut, labored or noisy breathing, diarrhea, red stains (oral, nasal, lacrimal and urinal), red stains on forepaws, urine perianal and brown-yellow ventrum stains, clear lacrimation and clear lacrimal stain. Gross Lesions: salivation, lacrimation reddened lungs and nasal stain.	III
Acute Dermal/Rat Mobay 91-022-JH Aug. 21, 1991 MRID 422563-14	LD_{50}: >2000 mg/kg (Local & Systemic) NOEL: <2000 mg/kg (Limit Test) Study Is Acceptable Toxic Signs: Urine stains, alopecia	III
Acute Inhalation/Rat Mobay 91-042-JZ Sep. 25, 1991 MRID 422563-16	LC_{50} Males: 2.650 mg/L Females: 2.750 mg/L Study is Acceptable Toxic Signs: ataxia, convulsions, hypoactivity, moribundity, nasal stain, urine stain and tremors, decrease body weight, death	III
Eye Irrit./Rabbit Mobay 91-335-JK Jun. 25, 1991 MRID 422563-18	Conclusion: Minimal Eye Irritation, resolved by 7 days Study is Acceptable Toxic Signs: Conjunctival redness, chemosis and discharge	III
Primary Dermal Irritation/ Rabbit Mobay 91-325-JG Aug. 15, 1991 MRID 422563-20	Conclusion: PIS: 1.08 (mildly-irritating) Study is Acceptable Toxic Signs: Erythema (grade 2) was observed in five animals, and edema (grade 1) was observed in one animal one hr following exposure. All resolved within 7 days.	IV
Dermal Sensitization/ Guinea pig Mobay 91-324-JC Aug. 23, 1991 MRID 422563-22	Conclusion: Not a Sensitizer Study is Acceptable Toxic Signs: None	N/A

TABLE 2C. SUMMARY OF ACUTE TOXICITY OF NTN 33893 40.7% FLOWABLE FORMULATION

TEST	RESULTS	CATEGORY
81-1 Acute Oral/Rat Study No. 9845-93 Date: March 25, 1993 MRID No.: 428577-02	LD50 Male 4687 mg/kg Female 4067 mg/kg Study is Acceptable Toxic Signs: Decreased body weight, clinical signs, and findings at necropsy	III
81-2 Acute Dermal/Rabbit Study No. 9846-93 Date: March 2, 1993 MRID No.: 428577-03	LD50 >5050 mg/kg (both sexes) Study is Acceptable Toxic Signs: Decreased body weight and body wt. gain in males	IV
81-3 Acute Inhalation/Rat Study No. 9847-93 Date: April 26, 1993 MRID No.: 422563-26	LC50 2.11 mg/L for males & females Study is Acceptable Toxic Signs: Decreased body weight gain, clinical signs & gross pathological findings.	III
81-4 Eye Irrit./Rabbit Study No. 9848-93 Date: Feb. 17, 1993 MRID No.: 428577-05	TIS: TIME 1hr 24hr 48hr 72hr IRRIT. SCORE 0.7 0.1 0.0 0.0 Study is Acceptable Toxic Signs: conjunctival redness	IV
81-5 Primary Dermal Irritation/Rabbit Study No. 9849-93 Date: Jan. 15, 1990 MRID No.: 422563-28	PIS: 0.0 Non-irritating. Study is Acceptable Toxic Signs: None	IV
81-6 Dermal Sensitization/Guinea pig Study No. 9850-93 Date: March 22, 1993 MRID NO. 428577-07	Conclusion: Not a Sensitizer Study is Acceptable Toxic Signs: None	N/A

B. SUBCHRONIC, CHRONIC, DEVELOPMENTAL, AND REPRODUCTIVE TOXICITY

Non-acute in vivo toxicity is summarized in the next table.

TABLE 3. SUMMARY OF NON-ACUTE TOXICITY NTN 33893

Guideline	Study Identification	Study Results
82-2	21-day Repeated Dose Dermal Species: Rabbit MRID: 422563-29 Core: Minimum DOC#: 009960	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.
83-4	28-Day Inhalation Study Species: Rat MRID: 422730-01 Core: Minimum DOC #: 010537	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.
83-1b	Chronic Species: Dog MRID: 422730-02 Core: Minimum DOC #s: 009960	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.
83-1a, 83-2a	Chronic/Onco Species: Rat MRIDs: 422563-31, 422563-32 Core: Minimum DOC #: 009960	NTN 33893 Technical was administered in the diet to 50 male and 50 female Bor WISW (SPF Cpb) rats per group at 0, 100, 300, 900 and 1800 ppm for 104 weeks. The 1800 ppm dose group tested in a separate study with its own concurrent controls. Results were as follows: The NOEL for Chronic Effects was 100 ppm (5.7 mg/kg/d in males, 7.6 mg/kg/d in females). The LOEL was 300 ppm based on increased thyroid lesions in males at 300 ppm (16.9 mg/kg/d) and above and in females at 900 ppm (73 mg/kg/d) and above; Decr. body wt. 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 males and/or females at 900 ppm (51.3 mg/kg/d in males, 73.0 mg/kg/d in females) or 1800 ppm. Oncogenicity: No apparent treatment-related effect at any dose.

Guideline	Study Identification	Study Results
83-2	2 Yr. Oncogenicity Species: Mouse MRID: 422563-35, 422563-36 Core: Minimum DOC #010537	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 (Two studies reviewed as one since dose levels were complementary). The NOEL was 1000 ppm (208 mg/kg/d in males, 274 mg/kg/d in females); the LOEL was 2000 ppm based on decreased body wt, decreased food consumption and decreased water intake. Oncogenicity: No apparent treatment-related effect at any dose.
83-3a	Developmental Toxicity Species: Rat MRID: 422563-38 Core: Minimum DOC #: 010537	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.
83-3b	Developmental Toxicity Species: Rabbit MRID: 422563-39 Core: Minimum DOC #s: 009960	NTN 33893 Technical was administered to 16 pregnant Chinchilla rabbits per group at 0, 8, 24, and 72 mg/kg/d during gestation days 6 through 19. The Maternal NOEL = 24 mg/kg/d; the LOEL = 72 mg/kg/d based on decreased food consumption; at 72 mg/kg/d, 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.
83-4	Reproductive Toxicity Species: Rat MRID: 422563-40 Core: Minimum DOC #010537	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.

C. MUTAGENICITY

Several mutagenicity studies are available. They are summarized on Table 4 below. Data requirements for these FIFRA TOX. Guidelines are satisfied by these submissions; no further studies need be submitted at this time.

Table 4.

Study Type (MRID No.)	Title (Report No.)	Reported Results	TB 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. 099676	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

Study Type (MRID No.)	Title (Report No.)	Reported Results	TB Evaluation
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
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

D. 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.

E. 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.

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.

Reviewed by: Myron S. Ottley, Ph.D. 5/23/94
Section IV, Tox. Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (H7509C) 5/24/94

DATA EVALUATION REPORT

STUDY TYPE:	**Acute Oral—Rat** (81-1)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	428557-02
TEST MATERIAL	GAUCHO 480
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid
STUDY NUMBER	9845-93
SPONSOR	Gustafson, Inc.
TESTING FACILITY	Stillmeadow, Inc., Sugar Land, TX 77478
TITLE OF REPORT	Acute Oral Toxicity Study in Rats
AUTHOR	Janice O. Kuhn, Ph.D.
REPORT ISSUED	March 25, 1993

CONCLUSIONS:

Gaucho 480 was administered once orally to 5 male and 5 female Sprague-Dawley rats per group at 4000, 4300 and 5050 mg/kg and observed for 14 days.

The LOEL was 4000 mg/kg for males and females, with the NOEL being less than 4000 mg/kg, the lowest dose level tested. Based on observations made during that time period, it is concluded that acute the **LD_{50} is 4687 mg/kg for males** with a dose-mortality slope of 1.086. **In females the LD_{50} is 4067 mg/kg**, with a slope of 1.067.

Tox. Category: III

Classification: Acceptable

This study satisfies the guideline requirements (81-1) for Acute Oral Toxicity on the Gaucho 480 formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** Gaucho 480 F.S.
   Description: Tan liquid. Lot No. F101:1-1 Ref. No. 4G4b;
   Purity: Not provided; Stability: Not specified, reported as "Stable".

2. **Test Animal:** Species: Rat, Strain: HSD:SD Age: young adult. Weight: Male—206-252 gm, Female—188-211 gm. Source: Harlan Sprague Dawley, Inc., Houston, Texas.

3. **Environment:** Rats were housed individually in suspended stainless steel cages suspended over bedding. Temperature: Not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Formulab Chow #5008 *ad libitum*; Water: tap *ad libitum* through automatic system.

METHODS

Animals were fasted for 16 hours prior to dosing. Groups of five male and five female rats received single doses of 4000, 4300, or 5050 mg/kg (3.30, 3.54, and 4.16 ml/kg respectively) of undiluted test material by oral intubation.

Observations for toxicity and mortality were made at least once daily for 14 days. Terminal body weights were taken just prior to treatment and days 7 and 14, or at time of death. Gross necropsy was conducted on each animal at termination of the study or at the time of discovery after death.

The method of sacrifice on day 14 after treatment was not specified.

The Quality Assurance statement was signed by Tracy Wooten on March 25, 1993.

RESULTS AND DISCUSSION

Mortality As seen in Table 1, Male and female deaths occurred during the study in a dose-related manner.

TABLE 1. MORTALITY FOLLOWING TREATMENT

Dose Level, mg/kg	Male (N=5)	Female (N=5)	Male & Female Combined (N=10)
4000	0	2	2
4300	1	4	5
5050	4	5	9

Clinical Signs Treatment-related signs of toxicity consisted of decreased activity, ataxia, body tremors, diarrhea, emaciation, nasal discharge, no defecation, piloerection, polyuria and ptosis. Most signs had completely disappeared by day 12.

Body Weight Body weight gain decreased in surviving animals in a dose-related manner from days 0 through 7.

Gross Necropsy Signs of diarrhea, lacrimation, nasal discharge, polyuria and salivation, discolored cecum, discoloration of the contents of the gastrointestinal tract, G.I. tract distended with gas, were observed in animals that died during the study or at terminal sacrifice. These findings were concluded to be treatment related.

Based on these results, it is concluded that acute the LD_{50} is 4687 mg/kg for males with a dose-mortality slope of 1.086. In females the LD_{50} is 4067 mg/kg, with a slope of 1.067. The LOEL was 4000 mg/kg for males and females, with the NOEL being less than 4000 mg/kg, the lowest dose level tested.

There were no major deficiencies in this study.

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Section IV, Tox. Branch I (7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (7509C) Marion Copley 5/24/94

DATA EVALUATION REPORT

STUDY TYPE:	**Acute Dermal Toxicity—Rabbit** (81-2)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	428577-03
TEST MATERIAL	GAUCHO 480
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid (proposed)
STUDY NUMBER	9846-93
SPONSOR	Gustafson, Incorporated
TESTING FACILITY	Stillmeadow, Inc. Sugar Land, TX 77478
TITLE OF REPORT	Acute Dermal Toxicity in Rabbits
AUTHOR	Janice O. Kuhn, Ph.D.
REPORT ISSUED	March 2, 1993

CONCLUSIONS:

GAUCHO 380 was administered once dermally to 5 male and 5 female New Zealand White rabbits per group at 5050 mg/kg and observed for 14 days.

No local effects were noted. The systemic NOEL for males was < 5050 mg/kg in males due to decreased body weight and body weight gain, and 5050 in females. Since no deaths occurred **the LD_{50} was >5050 mg/kg for both sexes.**

Tox. Category: IV

Classification: Acceptable

This study satisfies the guideline requirements (81-2) for Acute Dermal Toxicity on the 75 WP-WS formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** GAUCHO 480
   Description: tan liquid. Lot No. F101:1-1, Reference No. 4G4b. Purity: 40% Imidocloprid a.i.
   Stability: Not specified. Reported as "Stable".

2. **Test Animal:** Species: Rabbit, Strain: New Zealand White; Age: three to six months; Weight: Male—2.625 - 2.850 kg, Female—2.500 - 2.800 kg. Source: Ray Nichols Rabbitry, Lumberton, Texas.

3. **Environment:** Animals were housed individually in stainless steel cages suspended over bedding. Temperature: Not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Rabbit Chow; Water: tap *ad libitum* through automatic system.

METHODS

Backs of animals were shaved the day prior to exposure. Groups of five male and five female rats received a dose of 5050 mg/kg of test substance, applied undiluted to at least 10% of the body surface on the shaven back. Two layers of surgical gauze was used to hold the test substance in contact with the skin. Non-irritating adhesive tape was used to hold the gauze in place. All items were removed 24 hr later, and the area was washed with room temp. water to remove as much residue as possible.

Observations for toxicity and mortality were made once daily for 14 days. Body weights were taken on days 0, 7 and 14 post treatment.

Animals were subjected to gross pathological examination after sacrifice on day 14 post treatment.

The quality assurance statement was signed by Tracy Wooten on March 2, 1993.

RESULTS AND DISCUSSION

No deaths occurred at 5050 mg/kg during this study, therefore LD_{50} estimates were not determined. No treatment-related clinical signs were observed. One male failed to gain weight, and another male lost weight.

Reporting deficiencies included the stability of the test material, and environmental conditions such as temperature, humidity and photoperiod. However, the stability of Imidacloprid has already been established in other studies of longer duration, and the absence of significant toxic effects suggests that environmental

conditions were consistent with good animal husbandry. Therefore these reporting deficiencies did not effect the Core grading of the study.

It is concluded that the LD_{50} is >5050 mg/kg by the dermal route in the New Zealand White Rabbit. The NOEL was <5050 mg/kg for both males, and 5050 mg/kg for females.

Reviewed by: Myron S. Ottley, Ph.D. 5/19/94
Section IV, Tox Branch I (7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (7509C) 5/24/94

DATA EVALUATION REPORT

STUDY TYPE:	**Inhalation -- Rat** (81-3)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	428557-04
TEST MATERIAL	GAUCHO 480
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid
STUDY NUMBER	9847-93
SPONSOR	Gustafson, Inc.
TESTING FACILITY	Stillmeadow, Inc., Sugar Land, TX 77478
TITLE OF REPORT	Acute Inhalation Toxicity Study in Rats
AUTHOR	Janice O. Kuhn, Ph.D.
REPORT ISSUED	April 26, 1993

CONCLUSIONS

GAUCHO 480 FS was administered for 4 hr once by inhalation to five male and five female Sprague-Dawley rats per group at analytically confirmed doses of 0, 1.77, 2.11 or 2.14 mg/L and observed for 14 days.

The calculated **LC_{50} was 2.11 mg/L** for males and females, with a **Toxicity Category of III.**

Classification: **Acceptable.**

This study satisfies the guideline requirements for an inhalation study in the rat (81-3) on the 480 FS formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** GAUCHO 480 FS.
   Description: Tan liquid. Lot No. F101:1-1 Ref. No. 4G4b;
   Purity: Not provided; Stability: Not specified, reported as "Stable".

2. **Test Animal:** Species: Rat, Strain: HSD:SD Age: young adult. Weight: Male—210-270 gm, Female—200-246 gm. Source: Harlan Sprague Dawley, Inc., Houston, Texas.

3. **Environment:** Rats were housed individually in suspended stainless steel cages suspended over bedding. Temperature: Not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Formulab Chow #5008 *ad libitum*; Water: tap *ad libitum* through automatic system.

METHODS

Aerosol Generation

At the low dose level (1.77 mg/L), the aerosol was generated by a pressure operated Spraying System Co. air atomizer which aspirated the test material directly from its container, and elutriated the resulting aerosol through a baffling chamber. The concentrated aerosol was then diluted with dried nd filtered air and drawn into the exposure chamber. At the higher levels (2.11 and 2.14 mg/L), the aerosol was generated by a pressure operated Spraying System Co. directly from its container into the inhalation chamber. Airflow into the chambers was maintained through the use of a calibrated critical orifice. The rate of air changes per hour was 18.1, 26.3 and 27.2 for low, mid and high dose levels, respectively. Airflow, temperature and humidity were measured at 30-minute intervals. Airflow was regulated to ensure a minimum of 19% oxygen content. Test material concentration was determined analytically on an hourly basis. Particle size was determined twice during the exposure period and at the end of the exposure period.

Exposure and Observations

Groups of five male and five female rats were exposed in a single 4-hour exposure to analytical concentrations of 0, 1.77, 2.11 or 2.14 mg/L of air. Mid- and high-dose animals were washed after exposure to remove excess ingestible test material on fur. All animals were observed for signs of toxicity or mortality frequently on the day of exposure, and at least once/day thereafter for 14 more days. Individual body weights were recorded just prior to exposure, and on days 3, 7, and 14 post exposure. On day 14 post exposure, all surviving animals were sacrificed and a complete gross necropsy was performed on each rat sacrificed at that time, and also on those that may have died during the course of the study.

RESULTS

Mortality

The following table shows dose levels and deaths:

Concentration (mg/L)	Number Dead / Number Treated		
	Males	Females	Males + Females
1.77	0/5	0/5	0/10
2.11	3/5	2/5	5/10
2.14	5/5	5/5	10/10

Clinical Signs

Observed at all levels were decreased activity, body tremors, nasal discharge, piloerection, polyuria, ptosis and salivation. In addition, staggered gait was observed at the mid-dose. Clinical signs were no longer evident by day three after exposure.

Body Weight Gain

At the low dose, two males and two females lost weight between Days 0 and 7. At the same level, one female lost weight between Days 0 and 14, on one female failed to gain weight between Days 7 and 14. At the mid-dose, two females lost weight between Days 0 and 7.

Gross Pathology

In the males and females that died during the study, discolored, swollen and undersized lungs were observed. No other effects were observed.

Particle Size (Table 2)

TABLE 2. AEROSOL PARTICLE SIZES AS MEASURED DURING THE 4 HR EXPOSURE PERIOD

Mean Concentration	Mass Median Aerodynamic Diameter (μm) (Time Approximate)		Geometric Standard Deviation (Time Approximate)	
	1¼ hr Distrib.	3¼ hr Distrib.	1¼ hr Distrib.	3¼ hr Distrib.
1.77 mg/L	5.290	3.882	2.118	2.266
2.11 mg/L	5.349	6.462	2.463	2.192
2.14 mg/L	5.806	5.952	2.424	2.894

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

DISCUSSION

GAUCHO 480 FS was acutely toxic to male and female rats at the concentrations tested, causing death in 15 of 30 of the treated animals, and transient clinical signs. The LC_{50} is calculated (with 95% confidence limits) to be 2.11 mg/L for males and females, with a Tox. Category of III. Summary Table 3 below gives full details.

TABLE 3. LC_{50} VALUES (WITH 95% CONFIDENCE LIMITS) OF GAUCHO 480 AEROSOL IN RAT.

	LC_{50} (mg/L)	95% Confidence Limits	Slope Function (S)	95% Confidence Limits
Males	2.11	2.09-2.12	1.01	1.01
Females	2.11	2.10-2.13	1.00	1.00
Combined	2.11	2.10-2.12	1.00	1.00-1.01

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Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 5/24/94
Section IV, Tox Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE:	**Primary Ocular Irritation—Rabbit** (81-4)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	428577-05
TEST MATERIAL	GAUCHO 480
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid
STUDY NUMBER	9848-93
SPONSOR	Gustafson Incorporated
TESTING FACILITY	Stillmeadow, Inc., Sugar Land, TX 77478
TITLE OF REPORT	Primary Eye Irritation Study in Rabbits
AUTHOR	Janice O Kuhn, Ph.D.
REPORT ISSUED	February 17, 1993

CONCLUSION:

GAUCHO 480 was introduced into the conjunctival sac of the left eye of three male and three female New Zealand White rabbits at 0.1 ml of undiluted test material/animal. The right eye served as control in each animal. Animals were observed for 72 hours.

Minimal eye irritation was observed in the form of conjunctival redness, resolved by 48 hours. Irritation was resolved in five of the six animals by 24 hours. Since effects were minimal, with no corneal involvement, **the Tox. Category is IV.**

Classification: **Acceptable**

This study satisfies the guideline requirements (81-4) for Primary Ocular Irritation on the 75 WP-WS formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** GAUCHO 480
   Description: tan liquid. Lot No. F101:1-1, Ref. No. 4G4b.
   Purity: 40% Imidacloprid. Stability: Reported only as "Stable".

2. **Test Animal:** Species: Rabbit (3 male + 3 female), Strain: New Zealand White; Age: 3 to 6 months; Weight: reported as not applicable; Source: Ray Nichols Rabbitry, Lumberton, Texas.

3. **Environment:** Animals were housed individually in stainless steel cages suspended over bedding. Temperature: Not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Rabbit Chow; Water: tap *ad libitum* through automatic system.

METHODS

One-tenth of a ml of undiluted test substance was placed into the conjunctival sac of the left eye of each of six adult male rabbits. The eye lids were held together for about one second. The right eye was not treated, and served as a control.

Rabbits were observed for signs of toxicity to the cornea, iris and conjunctivae according to the Draize method. Lacrimation was also assessed. Observations were made 1 hr, 24 hr, 48 hr and 72 hr post treatment.

The quality assurance statement was signed by Tracy Wooten on Feb. 17, 1993.

RESULTS AND DISCUSSION

The cornea and iris were not adversely affected in any of the animals. As seen in Table 1, there was conjunctival redness (grade 2) in all six animals. All redness had resolved by 24 hours, except for slight redness (grade 1) in one male that was present at 24 hours but resolved by 48 hours.

Non-ocular lesions or other signs of toxicity were not observed. The test substance is considered a minimal eye irritant with a Toxicity Category of IV.

TABLE 1 RESULTS OF EYE IRRITATION TEST

Animal No./Sex	Time Post Dosing	Conjuntiva		
		Redness	Chemosis	Discharge
4094/M	1 hr	2	0	0
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
4096/M	1 hr	2	0	0
	24 hr	1	0	0
	48 hr	0	0	0
	72 hr	0	0	0
4098/M	1 hr	2	0	0
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
4095/F	1 hr	2	0	0
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
4097/F	1 hr	2	0	0
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
4099/F	1 hr	2	0	0
	24 hr	0	0	0
	48 hr	0	0	0
	72 hr	0	0	0
TOTAL AVERAGE SCORES	1 hr	2.0	0.0	0.0
	24 hr	0.2	0.0	0.0
	48 hr	0.0	0.0	0.0
	72 hr	0.0	0.0	0.0

SUMMARY OF RESULTS

TIME (hour, day)	1 hr	24 hr	48 hr	72 hr
IRRITATION SCORE	0.7	0.1	0.0	0.0

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Section IV, Tox. Branch I (7509C) Marion Copley 5/24/94

DATA EVALUATION REPORT

STUDY TYPE:	**Dermal Irritation—Rabbit** (81-5)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	~~428557-02~~ 428577-06
TEST MATERIAL	GAUCHO 480
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid
STUDY NUMBER	9849-93
SPONSOR	Gustafson,. Inc.
TESTING FACILITY	Stillmeadow, Inc., Sugar Land, TX 77478
TITLE OF REPORT	Primary Dermal Irritation Study in Rabbits
AUTHOR	Janice O. Kuhn, Ph.D.
REPORT ISSUED	February 16, 1993

CONCLUSION:

GAUCHO 480 was administered for 4 hr once dermally to shaved backs of three male and three female New Zealand White rabbits at 0.5 ml/animal of undiluted test substance, and observed for 72 hours.

Erythema/Eschar nor Edema were observed at any time on any of the test animals. **PIS: 0.00 (non-irritating). The Toxicity Category is IV.**

Core Classification: **Acceptable**

This study satisfies the guideline requirements (81-5) for Primary Dermal Irritation on the 75 WP-WS formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** GAUCHO 480 FS
   Description: tan liquid. Lot No. F101:1-1 Reference No. 4G4b
   Purity: 40%
   Stability: Not specified. Reported at "stable".

2. **Test Animal:** Species: Rabbit, Strain: New Zealand White; Age: three to six months; Weight: Not specified; Source: Ray Nichols Rabbitry, Lumberton, Texas.

3. **Environment:** Animals were housed individually in stainless steel cages suspended over bedding. Temperature: Not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Rabbit Chow; Water: tap *ad libitum* through automatic system.

METHODS

The backs and sides of three male & three female rabbits were shaved to expose 8 cm square the day prior to treatment. Half an ml mg of the test substance was applied undiluted to an area measuring 2.5 cm by 2.5 cm and secured with gauze and hypoallergenic tape; the rabbits trunk was wrapped loosely with a semi-permeable dressing to retard evaporation of volatiles, and to prevent possible ingestion of the test material. All materials were removed approximately 4 hr after treatment. The treated area was cleaned to remove as much residue as possible.

Animals were observed for signs of erythema and edema formation 1 hr, 24 hr, 48 hr, and 72 hr post dosing; findings were recorded in harmony with the Draize method.

The quality assurance statement was signed by Stacy Whooten on Feb. 16, 1993.

RESULTS AND DISCUSSION

Erythema and edema were not observed at any observation time throughout the study. A Primary Irritation Index of 0.00 was obtained. No lesions or other toxic signs were observed. GAUCHO 480 can be classified in Toxicity Category IV (non-irritating) for dermal irritation.

Reviewed by: Myron S. Ottley, Ph.D. MSOttley 5/23/94
Section IV, Tox. Branch I (7509C)
Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox Branch I (7509C) MarionCopley 5/24/94

DATA EVALUATION REPORT

STUDY TYPE:	**Dermal Sensitization—Guinea Pig** (81-6)
PC NO.	129099
TOX. CHEM NO.	497E
MRID NO.	428577-07
TEST MATERIAL	GAUCHO 480 FS
SYNONYMS	1-[(Chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine Imidachloprid (proposed)
STUDY NUMBER	9850-93
SPONSOR	Gustafson, Inc.
TESTING FACILITY	Stillmeadow, Inc., Sugar Land, TX 77478
TITLE OF REPORT	Dermal Sensitization Study in Guinea Pigs
AUTHOR	Janice O. Kuhn, Ph.D.
REPORT ISSUED	March 22, 1993

CONCLUSION:

GAUCHO 480 FS was administered to shaved backs of 10 DHPW guinea pigs (5 male / 5 female) at 0.4 ml of undiluted test substance per animal, following the induction/sensitization protocol. One week prior to the topical induction, intradermal induction was performed with 3 1 ml injections/animal. The results indicated that GAUCHO 480 FS is **Not a Sensitizer** by the modified Buehler method.

Core Classification: **Acceptable**

This study satisfies the guideline requirements (81-6) for Dermal Sensitization on the GAUCHO 480 FS formulation, and is acceptable for regulatory purposes.

MATERIALS

1. **Test Compound:** GAUCHO 480 FS
   Description: tan liquid. Lot No. F101:1-1, Reference. No.4G4b
   Purity: 40%
   Stability: Not specified, reported as "Stable".

2. **Test Animal:** Species: Guinea Pig (male), Strain: Hartley albino; Age: not specified; Weight: Males—390-465 gm, Females—335-400 gm; Source: Gustafson, Inc., Dallas, Texas.

3. **Environment:** Animals were housed 1 - 4 per cage (one sex per cage) in suspended stainless steel. Temperature: not specified; Humidity: not specified; Photoperiod: not specified; Food: Purina Guinea Pig Chow *ad libitum*; Water: tap *ad libitum*.

METHODS

Using the Buehler Topical Closed-Patch technique, a 0.4 ml volume of a undiluted test substance was applied to a 3.8 cm by 5 cm Coverlet Adhesive dressing, fixed to shaved area of guinea pig backs. The test groups were as follows:

Treatment Group	Number of Animals (Males/Females)
GAUCHO 480 FS -- Induction and Challenge	10 (5/5)
Control -- Challenge Only	10 (5/5)

★ applied at an undiluted volume of 0.4 ml.

Animals in the GAUCHO 480 FS test group received three topical induction applications (6-hr duration) on days 1, 8 and 15 of the study, followed by a topical challenge application (24 hr duration) on day 29. Animals in the Control group received only a single 24-hr application on day 29. Different areas of the shaved back were used for the three induction applications, the challenge dose. At the end of the exposure period, the bandages and pad were removed and the dose site was wiped clean using a dampened paper towel.

A concurrent positive control group was not used since positive controls are periodically tested in the lab and the last test was conducted six months prior to this study. Positive control substance was 1-chloro-2,4-dinitrobenzene at a concentration of 0.06% w/v in 95% ethanol.

Dermal irritation scores were determined approximately 24 and 48 hr after unwrapping for each induction a challenge treatment. After the challenge dose, the dose site and naive area were shaved for scoring irritation.

Body weights were recorded for all animals on days 0 and 28.

The quality assurance statement was signed by Tracy Wooten on March 22, 1993.

RESULTS AND DISCUSSION

Guinea pigs evaluated 24 and 48 hr after challenge dose showed no sensitization (induction or challenge) response to NTN 33893. Animals treated only with a challenge dose of NTN 33893 also gave no response.

There was no mortality. Mean body weight gain for GAUCHO 480 FS test animals was 142 g, compared with 130 g for non-induced control animals.

It is concluded that NTN 33893 75 WP-WS is not a dermal sensitizer in the guinea pig.

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