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

009960

JAN 11 1993

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Subject: I.D. Nos. 003125-URU, 003125-URL, 003125-URI, 003125-URT, 003125-URA: NTN 33893. Evaluation of Toxicity Data Submitted and Identification of Outstanding Toxicology Data Requirements

Tox. Chem. No. 497E
PC Code No. 129099
DP Barcode Nos. D179336, D179359, D179336, D179372, D179382
Submission Nos. S419490, S419541, S419548, S419552, S419557

From: Myron S. Ottley, Ph.D.
Section IV, Toxicology Branch I
Health Effects Division (H7509C)
Myron S. Ottley 1/8/93

To: Portia Jenkins/Dennis Edwards, Jr. (PM19)
Registration Division (H7508W)

Through: Marion P. Copley, D.V.M., D.A.B.T.
Section Head
Section IV, Toxicology Branch I
Health Effects Division (H7509C)
Marion Copley 1/8/93

Through: Karl Baetcke, Ph.D.
Branch Chief
Toxicology Branch I
Health Effects Division (H7509C)
Karl P. Baetcke 1/11/93

I. CONCLUSION

The submitted toxicity studies on NTN 33893 Technical and the 75%, 24%, 2.5% and 0.62% Formulations to support registration for non-food use have been reviewed and found to be **acceptable** for regulatory purposes. A Toxicity Profile of NTN 33893 is also included in this memo.

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Referral: 009960

1. OREB — Yes
2. Dietary Exposure — No

In order to provide a MOE of greater than 100, **actual daily exposure** would have to be less than 0.24 mg/kg/day based on the acute NOEL of 24 mg/kg/day for the developmental rabbit study (DER Attached).

In order to provide a MOE of greater than 100, **chronic exposure** would have to be less than 0.057 mg/kg/day based on the chronic NOEL of 5.7 mg/kg/day for the chronic rat study (DER attached).

II. ACTION REQUESTED

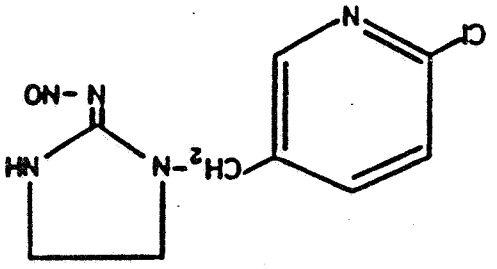
TB-1 received for evaluation the several studies required to fulfill data requirements for registration of NTN 33893 for non-food use. These data were submitted by Bayer AG.

III. PRODUCT INFORMATION

NTN33893,1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine [Imidacloprid—proposed] is a nitroquanidine insecticide, to be used on indoor and outdoor ornamental plants to control several insect pests such as adelgids, aphids, elm leaf beetle, lacebugs, and others. The proposed name is Imidacloprid. No food use registrations are existing at this time and there are no established tolerances for NTN 33893 in any plant or animal commodities. Technical NTN 33893 (003125-URU) is 94% pure, with 6% inerts. The registered products will be supplied as follows:

Concentrate: 75% (003125-URL)
23% (003125-URI)
Granular: 2.5% (003125-URT)
0.62% (003125-URA)

NTN 33893 has a molecular weight of 255.66. Its chemical structure is shown below:



The physical and chemical properties of NTN 33893 are listed below:

Color:	Light Yellow	
Physical State:	powder	
Odor:	Not Specified	
Solubility:	Water:	0.58 g/l @ 20°C
	Acetone:	20-50 g/l @ 20°C
	Acetonitrile:	20-50 g/l @ 20°C
	Dichloromethane:	50-100 g/l @ 20°C
	DMF:	>200 g/l @ 20°C
	DMSO:	>200 g/l @ 20°C
	n-Hexane:	<0.1 g/l @ 20°C
	Toluene:	0.5-1 g/l @ 20°C
	2-Propanol:	1-2 g/l @ 20°C
Stability:		
CAS Registry Number:		
Tox Chem Number:	497E	
PC Number:	129059	

IV. REQUIREMENTS (CFR 158.135) for Non-Food Use January 8, 1993

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)	N[1]	-	N	-
82-1	Oral Subchronic (Non-Rodent)	N[1]	-	N	-
82-2	21-Day Dermal	Y	Y	N	-
82-3	90-Day Dermal	N[2]	-	N	-
82-4	90-Day Inhalation	N	-	N	-
82-5	90-Day Neurotoxicity (hen)	N	-	N	-
82-6	90-Day Neurotoxicity (mammal)	N	-	N	-
83-1	Chronic Toxicity (Rodent)	N	-	N	-
83-1	Chronic Toxicity (Non-rodent)	N	-	N	-
83-2	Oncogenicity (Rat)	N	-	N	-
83-2	Oncogenicity (Mouse)	N	-	N	-
83-3	Developmental Toxicity (one species)	Y	-	N	-
83-3	Developmental Toxicity (two species—rodent & non-rodent)	N	-	N	-
83-4	Reproduction	N	-	N	-
83-5	Chronic/Oncogenicity	N	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	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	-

Legend Y = yes N = no

§ 75%, 23.1%, 2.5% and 0.62% Formulations

[1] Not Required because adequate chronic data available.

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

V. TOXICOLOGY PROFILE

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Technical NTN 33893

Guideline	Study; Company; Date; MRID #; Category; Classification	Study Results
81-1	Acute oral LD50 Species: rat Bayer AG Instit. Fur Tox. Germ Study#: T 2033060 MRID: 420553-31 Date: 12/15/89 CORE - ACCEPTABLE DOC#s: 009375	Male Sprague-Dawley rats dosed at: 0, 50, 100, 250, 315, 400, 450, 500 & 1800 mg/kg. Females dosed: 0, 100, 250, 315, 400, 475, 500, and 1800 mg/kg. LD50 (M) = 424 mg/kg (calculated). F > 450, < 475 mg/kg (estimated). Toxicity category II
81-2	Acute Dermal LD50 Species: rat Mobay Chem. Study#: T 5033063 MRID: 420553-32 Date: 11/15/89 CORE - ACCEPTABLE DOC#s: 009375	Sprague-Dawley rats dosed at 0 and 5000 mg/kg.n LD50 > 5000 mg/kg (limit test). Necropsy Observations: None Toxicity category IV
81-3	Acute inhalation LC50 Species: rat Bayer AG Instit. Fur Tox. Germ Study#: 16777 MRID: 420553-33 42281-01 Date: 06/06/88 CORE - = ACCEPTABLE DOC#s: 009375 New Document DER Attached	Wistar rats dosed at 69 mg/m3 aerosol, 1220, 2577, and 5323 dust. Control received conditioned air or 20,000 uL Lutrol vehicle. LC50 > 5323 mg/m3 (Tentative). upgraded Toxicity category IV
81-4	Primary eye irritation Species: rabbit Bayer AG Instit. Fur Tox. Germ Study#: T 8025515 MRID: 420553-34 Date: 02/25/89 CORE - ACCEPTABLE DOC#s: 009375	NZW rabbits given 0.1 mL of test substance in one eye. TIS: Primary Irrit. Index = 0. Non-irritating. Minimal redness (1 animal & swelling (1 animal) observed 1 hr. post-dosing; was completely gone at 24 hrs. Toxicity category IV
81-5	Primary dermal irritation Species: rabbit Bayer AG Instit. Fur Tox. Germ Study#: T 8025515 MRID: 420553-35 Date: 02/25/88 CORE - ACCEPTABLE DOC#s: 009375	4 hr dermal exposure to NZWrabbits at 500 mg/kg. PIS = 0.0 (non-irritating). Toxicity category IV

Technical (Cont.)

Guideline	Study Identification	Study Results
81-6	Dermal sensitization Species: guinea pig Bayer AG Instit. Fur Tox. Germ Study#: T 902561 MRID: 420553-36 Date: 03/15/88 CORE - ACCEPTABLE DOC#s: 009375	Not a sensitizer to DHPW guinea pigs.

NTN 33893 Technical

Guideline	Study Identification	Study Results
82-2	21-day Repeated Dose Dermal Species: Rabbit Bayer AG Dept. of Toxicology Study #: T 7029592 MRID: 422563-29 Date: June 11, 1990 Core: Minimum DOC#s: DER Attached	NTN 33893 Technical was administered at 1000 mg/kg to shorn backs of 5 male and 5 female New Zealand White rabbits for 6 hours/day, 5 days/week for 3 weeks. NOEL Systemic: 1000 mg/kg/day Dermal: 1000 mg/kg/day LOEL Systemic: >1000 mg/kg/day Dermal: >1000 mg/kg/day
83-1b	Chronic Species: Dog RCC, Research & Consulting Co. Study #: 100015 MRID: 422730-02 Date: Oct. 19,1989 Core: Minimum DOC #s: DER Attached	NTN 33893 Technical was administered in the diet to 4 male and 4 female Beagle dogs per group at 0, 200, and 1250 (increased to 2500 from week 17 onwards) ppm for 52 weeks. NOEL: 1250 ppm (41 mg/kg/d) LOEL: 2500 (72 mg/kg/d) Increased Cytochrome P-450 levels in males and females. Considered a threshold dose. 5000 ppm caused 50% mortality in rangefinding study.
83-1a, 83-2a	Chronic/Onco Species: Rat Bayer AG Study #: 100652 101931 MRIDs: 422563-31 422563-32 Dates: July 14, 1989, Aug 19, 1991 Core: Minimum DOC #s: DER Attached	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. NOEL: Chronic Effects: 100 ppm (5.7 mg/kg/d in males, 7.6 mg/kg/d in females) LOEL: Chronic Effects: 300 ppm 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.
83-3	Developmental Toxicity Species: Rabbit RCC, Research & Consulting Co. Study #: 083518 MRID: 422563-[illegible] Date: Jan. 8, 1992 Core: Minimum DOC #s: DER Attached	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. Maternal NOEL 24 mg/kg/d LOEL 72 mg/kg/d. Decreased food consumption; at 72 mg/kg/d: decreased body weight, increased resorption, increased abortion, and death. Developmental NOEL 24 mg/kg/d LOEL 72 mg/kg/d. Decrease body weight, increased skeletal abnormalities.

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NTN 33893 Technical — Mutagenicity Study Evaluation

DERs to be submitted with subsequent action

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 mg/kg	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/kg	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, a non-toxic dose	UNACCEPTABLE
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/kg	UNACCEPTABLE
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 mg/ml -S9 and 2000 mg/ml +S9, both toxic doses	ACCEPTABLE
Other genotoxicity (472563-50)	"Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells," Report No. 099676	Negative, but only tested up to 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

NTN 33893 75% Formulation

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Guideline	Study Identification	Study Results
83-1	Acute Oral LD50 Species: Rat Mobay Corp. Study #: 91-012-JJ MRID: 422563-12 Date: August 27, 1991 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was administered once by gavage to Sprague-Dawley rats (5/sex/dose) at 0, 1063, 2180, and 3170 mg/kg for males, and 0, 1063, 2180, 2750, and 3170 mg/kg for females. Animals were observed for 14 days LD50 Male 2591 mg/kg (calculated) Female 1858 mg/kg (calculated) Toxicity Category: III
81 2	Acute Dermal LD50 Species: Rat Mobay Corp. Study #: 91-022-JH MRID: 422563-14 Date: August 21, 1991 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was administered once dermally for 24 hr to Sprague-Dawley rats (5/sex/dose) at 0 and 2000 mg/kg. Animals were observed for 14 days. LD50 >2000 mg/kg Toxicity Category: III
81-3	Acute Inhalation Species: Rat Mobay Corp. Study #: 91-042-JZ MRID: 422563-16 Date: September 25, 1991 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was administered as a liquid aerosol by inhalation once for 4 hr to Sprague-Dawley rats (6/sex/dose) at 0, 2110, 2810, and 2990 mg/m3. Animals were observed for 14 days. LC50 Male: 2650 mg/m3 (calculated) Female: 2750 mg/m3 (calculated) NOEL <2110 mg/m3 LOEL 2110 mg/m3 Toxicity Category: III
81-4	Eye Irritation Species: Rabbit Mobay Corp. Study #: 91-335-JK MRID: 422563-18 Date: June 25, 1992 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was introduced into the conjunctival sac of the left eye of 6 male New Zealand White rabbits at 0.1 ml (44-46 mg). The right eye of each animal served as control. Animals were observed for 14 days. TIS: TIME 1hr 24hr 48hr 72hr 7d 14d IRRIT. SCORE 2.5 1.1 1 0.1 0 0 Toxicity Category: III
81-5	Primary Dermal Irritation Species: Rabbit Mobay Corp. Study #: 91-335-JG MRID: 422563-20 Date: August 15, 1991 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was administered for 4 hr once dermally to shaved backs of six male New Zealand White rabbits at 500 mg/animal, and observed for 7 days. PIS: 1.08 Mild irritation at 72 hr. Toxicity Category: IV
81-6	Dermal Sensitization Species: guinea pig Mobay Corp. Study #: 91-324-JC MRID: 422563-22 Date: August 23, 1991 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 75% Formulation was administered, in 3 6-hr topical induction applications followed by one 24-hr topical challenge 14 days later, to shaved backs of 15 Hartley albino guinea pigs. Conclusion: Not a Sensitizer

NTN 33893 240 F.S.

Guideline	Study Identification	Study Results
83-1	Acute Oral LD50 Species: Rat Mobay Corp. Study #: 89-012-DV MRID: 422563-13 Date: Feb. 26, 1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was administered once by gavage to Sprague-Dawley rats (5/sex/dose) at 0, 1030, 2100, 3595 and 4870 mg/kg for males, and 0, 2100, 3595 and 4870 mg/kg for females. Animals were observed for 14 days. LD50 Male >4870 mg/kg Female 4143 mg/kg (calculated Toxicity Category: III
81-2	Acute Dermal LD50 Species: Rat Mobay Corp. Study #: 89-025-EB MRID: 422563-15 Date: February 22, 1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was administered once dermally for 24 hr to New Zealand White rabbits (5/sex/dose) at 0 and 2000 mg/kg. Animals were observed for 14 days. LD50 >2000 mg/kg Toxicity Category: III
81-3	Acute Inhalation Species: Rat Mobay Corp. Study #: 89-042-EG MRID: 422563-17 Date: February 27,1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was administered as a liquid aerosol by inhalation once for 4 hr to Sprague-Dawley rats (6/sex/dose) at 0, 5060, and 5330 mg/m3. Animals were observed for 14 days. LC50 >5330 mg/m3 NOEL <5060 mg/m3 LOEL 5060 mg/m3 Toxicity Category: IV
81-4	Eye Irritation Species: Rabbit Mobay Corp. Study #: 89-335-DZ MRID: 422563-19 Date: January 15,1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was introduced into the conjunctival sac of the one eye of 6 New Zealand White rabbits (3/sex) at 0.1 ml. The other eye of each animal served as control. Animals were observed for 14 days. TIS: TIME 1hr 24hr 48hr 72hr 7d 14d IRRIT. SCORE 1.0 0.3 0. 0.0 0 0 Toxicity Category: III
81-5	Primary Dermal Irritation Species: Rabbit Mobay Corp. Study #: 89-325-DU MRID: 422563-21 Date: January 15, 1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was administered for 4 hr once dermally to shaved backs of six New Zealand White Rabbits (3/sex) at 500 mg/animal, and observed for 7 days. PIS: 0.0 Non-irritating. Toxicity Category: IV
81-6	Dermal Sensitization Species: Guinea pig Mobay Corp. Study #: 89-324-DO MRID: 422563-23 Date: February 22, 1990 Core: Minimum DOC #: DER to be submitted with subsequent action	NTN 33893 240 F.S. was administered, in 3 6-hr topical induction applications followed by one 24-hr topical challenge 14 days later, to shaved backs of 15 Hartley albino guinea pigs. Conclusion: Not a Sensitizer

NTN 33893 2.5% Granular 009960

Guideline	Study Identification	Study Results
81-1	Acute oral LD50 Species: rat Mobay Chem. Study#: 89-012-DY MRID: 420553-24 Date: 02/26/90 CORE - ACCEPTABLE DOC#s: 009375	LD50 > 4820 mg/kg (5000 mg/kg nominal, limit test) Necropsy Observations: None. Toxicity category IV
81-2	Acute Dermal LD50 Species: rabbit Mobay Chem. Study#: 89-025-DS MRID: 420553-25 Date: 01/15/90 CORE - ACCEPTABLE DOC#s: 009375	NZW rabbits dose at 0 and 2000 mg/kg. LD50 > 2000 mg/kg. Necropsy: None Toxicity category III
81-3	Acute inhalation LC50 Species: rat Mobay Chem. Study#: 89-042-DX MRID: 420553-26 Date: 02/26/90 CORE - ACCEPTABLE DOC#s: 009375 PER Attached	Sprague-Dawley rats dosed at 0 and 5092 mg/m3. LC50 > 5092 mg/m3 (95% C.L. intervals) Tentative. Necropsy: None Data submission is incomplete. Verification of particle size & distribution in exposure chamber not possible. See deficiencies section. upgraded. Toxicity Category IV
81-4	Primary eye irritation Species: rabbit Mobay Chem. Study#: 89-335-DT MRID: 420553-27 Date: 01/15/90 CORE - ACCEPTABLE DOC#s: 009375	NZW rabbits received 0.1 mL of pulverized test substance/animal. Reversible irritation by 14 days. TIS Time 1 hr 24 hr 48 hr 72 hr 7 d 14 d Iris Irrit Score 2.3 1.2 1.0 0.5 0.2 0.0 Toxicity Category II
81-5	Primary dermal irritation Species: rabbit Mobay Chem. Study#: 89-325-ED MRID: 420553-28 Date: 12/11/90 CORE - ACCEPTABLE DOC#s: 009375	4 hr dermal exposure to NZW rabbits at 50 mg/animal & observed for 72 hrs. PIS = 0.0. Nonirritating. Toxicity Category IV

2.5% granular (cont.) 009960

Guideline	Study Identification	Study Results
81-6	Dermal sensitization Species: guinea pig Mobay Chem. Study#: 89-324-DN MRID: 420553-29 Date: 12/11/90 CORE - ACCEPTABLE DOC#s: 009375	Not a sensitizer to Hartley guinea pigs.

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NTN 33893 0.62% Granular

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Guideline	Study Identification	Study Results
81-1	Acute oral LD50 Species: rat Mobay Chem. MRID#: 420553-23 Date: 09/30/91 DOC#s: 009375	Study waived. Use data from study #89-012-DY (MRID 420553-24). Toxicity Category IV
81-2	Acute Dermal LD50 Species: Mobay Chem. MRID#: 420553-23 Date: 09/30/91 DOC#s: 009375	Study waived. Use data from study #89-025-DS (MRID 420553-25). Toxicity Category III
81-4	Primary eye irritation Species: rabbit Mobay Chem. MRID#: 420553-23 Date: 09/30/91 DOC#s: 009375	Study waived. Use data from study #89-335-DT (MRID 420553-27) Toxicity Category II
81-5	Primary dermal irritation Species: Mobay Chem. MRID#: 420553-23 Date: 09/30/91 DOC#s: 009375	Study waived. Use data from study #89-325-ED (MRID 420553-28) Toxicity Category II
81-6	Dermal sensitization Species: Mobay Chem. MRID#: 420553-23 Date: 09/30/91 DOC#s: 009375	Study waived. Use data from study #89-324-DN (MRID 420553-29) Not a sensitizer.

VI. DATA GAPS

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No data gaps have been identified for the requested new registrations for NTN 33893 and formulations in this action.

VII. ACTION TAKEN TO REMOVE DATA GAPS AND OBTAIN ADDITIONAL INFORMATION

Not Applicable

VIII. REFERENCE DOSE (RfD)

This is a non-food use registration. The Rfd will be set subsequently as part of the food-use Registration Application.

IX. PENDING REGULATORY ACTIONS

Tox. Branch I is not aware of any regulatory actions against NTN 33893.

X. TOXICOLOGICAL ISSUES

Concern had been raised for WAK 3839, a trace (30 ppm) by-product of NTN, and an established mammalian metabolite, since this chemical is a nitrosamine. Testing in the Mutagenicity battery of assays provided no support for this concern. In addition, the submitter reported that in the rat oncogenicity study the concentration of WAK 3839, as a metabolic by-product of NTN 33893, accounted for 7% of the 1800 ppm administered dose level. Since this level, which is approximately 2,300 times higher than the impurity level of NTN 33893, produced no evidence of oncogenicity, the related concerns for this nitrosamine impurity appear to have been met. (See also Attachment #1.)

XI. OTHER

The summary memorandum on the Mutagenicity of NTN 33893 from Irving Mauer to Myron Ottley, dated Jan. 7, 1993 will be submitted with a subsequent EUP for this chemical. Additional studies (not required for this non-food use registration) with this action will be reviewed for the food use application.

Myron. Tox chem #497E

Attachment #1

TO THE FILE

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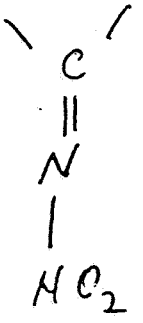
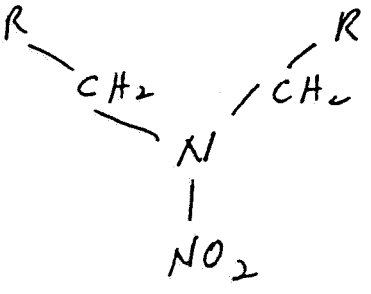
Mobay Corporation's data show that neither NTN-33893 nor its impurity/metabolite WAK-3839 has mutagenic/carcinogenic activity in laboratory testing systems. Testing had been done in anticipation of concern for the nitrosoamine moiety in WAK-3839.

Dr. Yin-Tak Woo of OTS/HERD has confirmed these negative findings on the basis of structure-activity considerations, stating that there is no alpha-hydrogen present on WAK 3839. As supported by Casarett & Doull (1975), at least one alkyl group must be attached to the nitrosamine to precipitate carcinogenicity.

There appears, therefore, to be insufficient basis to support concern for mutagenic/carcinogenic potential for NTN-33893 or its impurity/metabolite WAK-3839, due to the nitrosoamine presence.



WA-3839



Nitrosoamine

Myron S. Ottley
April 10, 1991

Reviewed by: Myron S. Ottley, Ph.D. MSOttley 12/29/92 009960
Toxicology Branch I, Section IV (H7509C)
Secondary review: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 12/29/92
Section Head, Toxicology Branch I, Section IV (H7509C)

DATA EVALUATION REPORT—Supplemental
Original DER: HED Doc. #009375

STUDY TYPE:	**Acute Inhalation Toxicity—RAT** (81-3)
TOX. CHEM NO:	497E
MRID NO.:	422861-02 (Supplemental to MRID #420553-26)
PC NO:	129099
TEST MATERIAL:	**NTN 33893** 2.5% Granular
SYNONYMS:	Imidacloprid (proposed) 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine
STUDY NUMBER:	89-042-DX
SPONSOR:	Bayer AG
TESTING FACILITY:	Miles Inc., Stilwell, Kansas
TITLE OF REPORT:	Acute Four-Hour Inhalation Toxicity Study with Bay NTN 33893 2.5% Granular in Rats
AUTHOR:	D.L. Warren
REPORT ISSUED:	February 26, 1990

CONCLUSION

Toxicity Category: IV

Classification: Core ~~minimum~~ Acceptable This study satisfies the guideline (81-3) requirements for an Inhalation Study in the rat, and is considered acceptable for regulatory purposes.

RESULTS AND DISCUSSION

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This document adequately responds to the concerns Toxicology Branch I had concerning the rat inhalation data submitted (MRID #420553-33), and upgrades that study to Core-minimum.

17

Reviewed by: Myron S. Ottley, Ph.D. MSOttley 12/29/92 009960
Toxicology Branch I, Section IV (H7509C)
Secondary review: Marion P. Copley, D.V.M., D.A.B.T. Marion Copley 12/29/92
Section Head, Toxicology Branch I, Section IV (H7509C)

DATA EVALUATION REPORT—Supplemental
Original DER: HED Doc. #009375

STUDY TYPE:	**Acute Inhalation Toxicity—RAT** (81-3)
TOX. CHEM NO:	497E
MRID NO.:	422861-01 (Supplemental to MRID #420553-33)
PC NO:	129099
TEST MATERIAL:	**NTN 33893** Technical
SYNONYMS:	Imidacloprid (proposed) 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine
STUDY NUMBER:	16777
SPONSOR:	Bayer AG
TESTING FACILITY:	Bayer AG Dept. of Toxicology, Wuppertal 1, West Germany
TITLE OF REPORT:	Study for Acute Inhalation Toxicity in the Rat in Accordance with OECD Guideline No. 403
AUTHOR:	Dr. J. Pauluhn
REPORT ISSUED:	June 6, 1988

CONCLUSION

Toxicity Category: IV

Classification: Core-Acceptable. This study satisfies the guideline (81-3) requirements for an Inhalation Study in the rat, and is considered acceptable for regulatory purposes.

009960

RESULTS AND DISCUSSION

This document adequately responds to the concerns Toxicology Branch I had concerning the rat inhalation data submitted (MRID #420553-33), and upgrades that study to Core Acceptable

Reviewed by: Myron S. Ottley, Ph.D. 12/28/92 009960
Toxicology Branch I, Section IV (H7509C)
Secondary review: Marion P. Copley, D.V.M., D.A.B.T.
Section Head, Toxicology Branch I, Section IV (H7509C) 12/28/92

DATA EVALUATION REPORT

STUDY TYPE:	21-Day Repeated Dose Dermal—Rabbit (82-2)
TOX. CHEM NO:	497E
MRID NO.:	422563-29
PC NO:	129099
TEST MATERIAL:	NTN 33893
SYNONYMS:	Imidacloprid (proposed) 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine
STUDY NUMBER:	T 7029592
SPONSOR:	Bayer AG
TESTING FACILITY:	Bayer AG Dept. of Toxicology, Wuppertal 1, West Germany
TITLE OF REPORT:	NTN Techn. Study for Subacute Dermal Toxicity in the Rabbit
AUTHOR:	Dr. W. Flucke
REPORT ISSUED:	June 11, 1990

CONCLUSION:

NOEL:	Systemic:	1000 mg/kg/day
	Dermal:	1000 mg/kg/day (limit dose)
LOEL:	Systemic:	> 1000 mg/kg/day
	Dermal:	> 1000 mg/kg/day

Classification: **Core-mininum**

This study satisfies the guideline (82-2) requirements for a 21-Day Repeated Dose

Dermal Study in the rabbit, and is considered acceptable for regulatory purposes.

A signed quality assurance statement was present.

A. MATERIALS

1. **Test compound**: NTN 33893 technical. Description: yellow powder. Batch #: 180587. Purity: 95.0%.

2. **Test animals**: Species: rabbit. Strain: HC:NZW New Zealand White. Age: 10 - 13 weeks at start of dosing. Weight: 2.93 - 3.09 kg, males; 22.65 - 3.43 kg, females. Source: Interfauna UK, Ltd., Huntingdon, England.

B. STUDY DESIGN

1. **Animal assignment**: After a 21-day acclimation period, animals were assigned randomly according to Table 1.

TABLE 1. GROUP ASSIGNMENT

Test Group	Dose on Skin mg/kg/d	Group Size	
		Male	Female
Vehicle Control[1]	0	5	5
Treatment	1000	5	5

2. **Environmental Conditions** All animals were housed individually in Cellidor Rabbit cages. Animals were fed Ssniff Spezialdiaten GmbH, Soest/Westfalen rabbit chow and tap water ad libitum. Ambient temperature: 22±2 °C; humidity: approx. 50%; light/dark cycle: 12 hours.

3. **Rationale for dose selection** The authors based their dose selection on the results of a preliminary study (T 4027690). These data were not provided. However, dosing was conducted up to the limit dose of 1000 mg/kg/day.

4. **Test Compound Preparation** NTN 33893 was made into a paste before each

[1]2% Cromophor EL in saline. Volume 1.5 ml/kg body weight.

application using a 2% (v/v) Cremophor EL solution in physiological saline solution.

5. **Compound Administration** Backs and flanks of rabbits were shorn 24 hours prior to first exposure, and twice weekly thereafter. The amount of NTN 33893 paste applied (1000 mg/kg body weight) was determined based on individual weekly body weight. The treated area (11 cm x 12 cm) was covered with muslin cloth folded into four, and secured with adhesive tape. Animals were exposed 6 hr/day, 5 days/wk. After exposure, the treated area was cleansed with soap and water.

6. **Compound Stability and Concentration** in vehicle: Analysis of test sample in the vehicle showed excellent stability and concentration consistency over a 24 hr period. The reported range was 1000±1.5 mg, 95.5% - 95.7%.

7. **Grading of Dermal Irritation** Dermal irritation was scored according to the method of Draize and erythema and edema were graded on scales of 0 - 4 for increasing severity.

8. **Statistics** The arithmetic group mean and standard deviation were calculated from the diet and body weights, as well as from the measured results obtained in the medical laboratory. The values of the test group were compared to the values of the corresponding control group using the two-tailed U Test according to Mann and Whitney, and Wilcoxon (reference citations).

C. METHODS AND RESULTS

1. Observations

Animals were inspected at least once daily for signs of toxicity and mortality. Treated skin sites were examined for signs of redness.

No treatment-related deaths occurred. One female in the control group was sacrificed on Day 3 of treatment due to a fractured femur. The animal was replaced on the next day of treatment (day 6).

No treatment-related changes in appearance or behavior were observed. No indications of dermal irritation were observed.

2. Food consumption

Individual food consumption was determined weekly beginning on day 7 of the study. Mean daily food intake was calculated from this data.

No treatment-related effects on food consumption were observed. At the end of Week 1 the mean consumption of treated males was 4% higher than controls ($p < 0.05$); however, this finding is of no toxicological significance.

3. **Body weight**

Animals were weighed weekly during the study, on days 0, 7, 14 and 22.

No treatment-related effects on body weight or body weight gain were observed.

4. **Blood** was collected at termination for hematological analysis (including differential leukocyte count) and clinical chemistry analysis from all animals.

CHECKED (X) parameters were examined.

a. **Hematology**

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

Results - No significant differences between control and treated animals were observed for any hematology parameter examined.

b. **Clinical Chemistry**

X		X	
	Electrolytes:		Other:
X	Calcium*		Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorous*		Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total serum Protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	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 significant differences between controls and treated animals were observed for

any clinical chemistry parameter examined. 009960

5. **Liver Tissue**

At necropsy on day 22, liver tissue from all animals was assayed for n-demethylase, o-demethylase, cytochrome P-450 and Triglycerides.

There were no significant differences between control and treated groups in N- and o- demethylase activity, triglycerides and cytochrome P-450. Treatment with NTN 33893 did not lead to induction of the examined enzyme systems.

6. **Sacrifice and Pathology**

All animals were weighed, anesthetized by the barbiturate Evipan®, exsanguinated and subjected to gross and microscopic pathological examination. The following organs and organ sections were collected for histological examination: treated and untreated skin, thyroids, lung, heart, liver, spleen, kidneys, adrenals, testes, epididymides, ovaries, uterus, sternum.

a. **Organ weights** (absolute and relative) for males and females were determined form brain, thyroids, heart, lung, liver, kidneys, adrenals, spleen, testes and ovaries.

No significant differences in absolute or relative liver or kidney weights were noted among any treatment groups.

b. **Gross Pathology** No apparent systemic, treatment-related macroscopic pathology was observed. No skin lesions or other signs of local dermal toxicity were observed.

c. **Microscopic pathology**

i) **Non-neoplastic** No treatment-related systemic histopathology was observed.

ii) **Neoplastic** No neoplastic lesions were observed.

D. DISCUSSION

Repeated dermal application on NTN 33893 technical, at a dose level of 1000 mg/kg, was tolerated by the rabbit with intact skin without local or systemic effects, including lesions. The NOEL for this study is 1000 mg/kg. A LOEL was not established.

Study deficiencies: In the Clinical Chemistry assays, lactic acid dehydrogenase, and cholesterol levels were not determined.

These deficiencies were not considered sufficient to alter the conclusions of the study.

CORE GRADE: **Core-minimum**

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Secondary reviewer: Marion P. Copley, D.V.M., D.A.B.T.
Section IV, Tox. Branch (H7509C) 12/29/92

DATA EVALUATION REPORT

STUDY TYPE: **Chronic/Onco -- Rat** (83-1, 83-2)

TOX. CHEM. NO.: 497E
PC NUMBER: 129099.
MRID Nos.:
1. 422563-31
2. 422563-32

TEST MATERIAL: **NTN 33893 Technical**

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

STUDY NUMBERs:
1. 100652
2. 101931

SPONSOR: Miles Corp.

TESTING FACILITY: Bayer AG, Dept. of Toxicology, West Germany

TITLEs OF REPORTs
1. Chronic Toxicity and Cancerogenicity [Carcinogenicity] Studies on Wistar Rats (Administration in the Feed over 24 Months)
2. Chronic Toxicity and Carcinogenicity Studies on Wistar Rats (Administration in the Feed over 24 Months) Supplementary MTD study for Two-Year Study (T 1025699)

AUTHOR(s):
1. Dr. R. Eiben, Dr. G. Kaliner
2. Dr. R. Eiben

REPORTs ISSUED:
1. July 14, 1989
2. August 19, 1991

CONCLUSION:

NOEL: Chronic Effects: 100 ppm (5.7 mg/kg/d in ♂, 7.6 mg/kg/d in ♀)

LOEL: Chronic Effects: 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 at any dose

CLASSIFICATION: **Core Minimum**. Although each study is considered Supplementary because of inadequate dose level complement, the combined data provide a dosing schedule that is consistent with guidelines.

These studies satisfy the guideline requirements (83-1, 83-2) for a Chronic/Onco Rat study on NTN 33893 Technical, and are acceptable for regulatory purposes.

A. MATERIALS

1. Test compound NTN 33893 Technical; Description: Solid—beige powder; Batch #: 180587; Purity: 94.3% - 95.3%.

2. Test animals Species: Rat; Strain: Bor WISW (SPF Cpb); Age: 5 - 6 weeks; Weight: 56 - 111 gm (males), 59 - 100 gm (females); Source: Winkelmann of Borchen.

B. STUDY DESIGN

1. Animal assignment Animals in both studies were fed NTN Technical in the diet daily for up to 24 months in dose groups shown in Table 1, and examined for signs of toxicity. They were assigned randomly as illustrated below.

Dose Group	Animals/Group		Conc. in Feed ppm	Average Intake, mg/kg/d* ♂/♀
	Main Study ♂/♀	Interim (52 wks) Sacrifice ♂/♀		
Control	50/50	10/10	0	0.0 / 0.0
Low Dose	50/50	10/10	100	5.7 / 7.6
Mid Dose	50/50	10/10	300	16.9 / 24.9
High Dose	50/50	10/10	900	51.3 / 73.0
Control**	50/50	10/10	9	0.0 / 0.0
High Dose**	50/50	10/10	1800	102.6 / 143.7

* Provided by Authors ** Supplementary Study

2. **Dose Selection** Dose levels were based on the results of two subchronic studies (98 and 96 days, resp.) with doses of 0, 150, 600 and 2400 ppm (MRID #422563-27) and 0, 120, 600, and 3000 ppm (MRID #422563-34).

   Doses of 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 weights 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 decreased 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 necroses were observed in males. At 3000 ppm degenerative testicular alterations were observed in males.

   Based on these findings, dose levels of 0, 100, 300 and 900 ppm were selected for the 2-year study. The companion study at 1800 ppm was subsequently conducted to reach an adequate dose level to test carcinogenic potential, since the 900 ppm levels failed to show adequate toxicity.

3. **Diet preparation** Diet was prepared prior to onset of the study and stored at room temperature. Assays for homogeneity, stability and concentration were conducted before test initiation and approximately each quarter thereafter.

4. **Food and Water Administration** Animals received food and water *ad libitum*.

5. **Statistics** The procedures utilized in analyzing the numerical data are attached.

6. **Quality Assurance** A signed quality assurance statement was attached.

C. METHODS AND RESULTS

1. **Clinical Observations** Animals were inspected at least twice daily (once on weekend days and holidays) for signs of toxicity and mortality. Detailed examination of individual animals was performed once weekly.

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

2. **Food and Water Consumption and Compound Intake** Food consumption was

measured weekly up to Week 19, and every four weeks thereafter. Mean daily diet consumption was calculated and compound intake were determined from the consumption and body weight gain data.

While food consumption decreased at 300 ppm and above relative to concurrent controls ($p < 0.05$ and/or ($p < 0.01$), these decreases were small, generally less than 10%, and no consistent pattern of variances emerged in any of the test groups, compared with corresponding controls.

No significant changes in water consumption were observed.

3. **Body weight** Animals were weighed prior to onset of treatment (Week 0), weekly through Week 14, at two-week intervals beginning at Week 16, and at terminal sacrifice.

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 to 8% in females) during Weeks 1 - 4,9 - 13, 18 and 22 (Table 2A). At 1800 ppm, male and female body weights were significantly less than their control group ($p < 0.01$), up to 12% in males or up to 11% in females throughout the study. Body weight gain data were not provided, however, Table 2B shows that body weight gain was at least 10% less than concurrent controls only in females at 900 ppm and 1800 ppm. The 18.5% difference in females at 900 ppm is not considered biologically significant, since a dose-response relationship was not observed. No difference in males was noted.

TABLE 2A. STATISTICALLY SIGNIFICANT DIFFERENCES IN BODY WEIGHT & BODY WEIGHT GAIN, COMPARED WITH CONCURRENT CONTROLS

Dose Level, ppm	Body Weights	
	Male	Female
Control		
100	n.s.	n.s.
300	n.s.	n.s.
900	up to 5%	up to 8%
Control		
1800	up to 12%	up to 11%

n. s. = not statistically significant

TABLE 2B. PERCENT BODY WEIGHT GAINS (>10%) OBSERVED IN FEMALES BETWEEN WEEKS 4 AND 13, AND WEEKS 0 AND 102, COMPARED WITH CONCURRENT CONTROLS

Dose Level	Week 4 — Week 13	Week 0 — Week 102
100 ppm	-22.2%	
300 ppm	-13.0%	
900 ppm	-18.5%	-11.2%
1800 ppm	-13.2%	-16.2%

In the low dose groups, body weights were similar to controls. Similarly, body weight gain was at least 10% less than controls only in females, at 900 ppm and above. Therefore, the NOEL for body weight is 300 ppm, and the LOEL is 900 ppm.

4. **Ophthalmological examination** Animals were examined at the start of the study. After 12 months, examinations were conducted on groups of 10 male and 10 female rats from the 900 ppm group and its controls, and on all 1800 ppm animals and their controls. The pupillary reflex of both eyes was tested first in a darkened room and the area around the eye and the anterior regions of the eye were assessed. After dilating the pupils with Mydriaticum-Roche™ eye-drops, the refractive sections of the eye and the fundus were examined with the aid of an indirect ophthalmoscope.

No dose-related effects on ophthalmological parameters were observed.

5. **Blood** was collected from all animals at the end of Weeks 5 and 13 in the main test group, and after Week 17 in the recovery group for hematology and clinical analysis. 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 dos-related effects on hematological parameters were observed

b. **Clinical Chemistry**

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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 and were generally within the range of normal variation.

6. **Urinalysis**

Urine was collected from fasted animals a few days prior to scheduled blood sampling and analysis (at the end of Weeks 5 and 13 in the main test group, and after Week 17 in the recovery group). The CHECKED (X) parameters were examined.

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

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

Sacrifice and Pathology

Rats were anaesthetized with ether and sacrificed by exsanguination. All animals that died and that were sacrificed on schedule were subject to gross pathological examination. The CHECKED (X) tissues were collected for histological examination, and additional tissues were retained.

X	Digestive system
X	Tongue
X	Salivary glands
X	Esophagus
X	Stomach
X	Duodenum
X	Jejunum
X	Ileum
X	Cecum
X	Colon
X	Rectum
XX	Liver
X	Gall bladder
X	Pancreas
	Respiratory
X	Trachea
XX	Lung
	Nose
	Pharynx
X	Larynx

X	Cardiovasc./Hemat.
X	Aorta
XX	Heart
X	Bone marrow
X	Lymph nodes
XX	Spleen
	Thymus
	Urogenital
XX	Kidneys
X	Urinary bladder
XX	Testes
X	Epididymides
X	Prostate
X	Seminal vesicle
XX	Ovaries
X	Uterus

X	Neurologic
XX	Brain
X	Peripheral Nerve
X	Spinal c. (3 levels)
X	Pituitary
X	Eyes (optic nerve)
	Glandular
XX	Adrenal gland
X	Lacrimal gland
X	Mammary gland
	Parathyroids
X	Thyroid gland
	Other
X	Bone
X	Skeletal Muscle
X	Skin
	All gross lesions and masses

a. **Organ weights** (See Table 3 or absolute changes, Table 4 for relative changes) At 900 ppm, male and female absolute liver weights 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 or 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. It appears that the organ weight toxicity is somewhat reversible at this dose level.

At 1800 ppm, male absolute spleen weights were decreased 17% ($p<0.01$) at 12 months. In female, absolute spleen weights were decreased 14% ($p<0.01$) at 24 months. Male absolute lung weight was also decreased by 17% ($p<0.01$) at 12 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. It appears that effects observed in males at 12 months were less severe at 24 months; females, however, were more widely and severely affected at 24 months than 12 months, indicating a cumulative effect of treatment.

No other significant changes in organ weights were observed at lower dose levels. The NOEL for organ weight changes is therefore 300 ppm; the LOEL is 900 ppm.

TABLE 3. ABSOLUTE ORGAN WEIGHT CHANGES OBSERVED AT INTERIM SACRIFICE (AFTER 12 MONTHS) AND AT TERMINATION (24 MONTHS)

Dose→	900 ppm				1800 ppm			
Sex→	Male		Female		Male		Female	
Time→	12 Mo.	24 Mo.	12 Mo.	24 Mo.	12 Mo.	24 Mo.	12 Mo.	24 Mo.
Liver	-14%*	+2%	-14%*	-4%*	-10%§	-7%*	-15%**	-17%**
Kidney			-13%*	-5%*				-11%**
Lung					-17%**	-7%		
Heart			-7%	-9%*			-10%	-15%**
Spleen					-17%**	-1%	-8%	-14%**
Adrenals							-3%	-17%**

§ Not statistically Significant * ($p<0.05$) ** ($p<0.01$)

TABLE 4. RELATIVE ORGAN WEIGHT CHANGES OBSERVED AT INTERIM SACRIFICE (AFTER 12 MONTHS) AND AT TERMINATION (24 MONTHS)

Dose→	900 ppm				1800 ppm			
Sex→	Male		Female		Male		Female	
Time→	12 Mo.	24 Mo.	12 Mo.	24 Mo.	12 Mo.	24 Mo.	12 Mo.	24 Mo.
Liver							-8%§	-6%*
Lung	+9%*	-7%*	+9%*	+3%				
Spleen	0%	-2%*						
Testis					+13%*	-3%		
Ovaries							-13%	+21%**
Brain			+12%**	+6%*			-5%	+11%**

§ Not statistically Significant * ($p<0.05$) ** ($p<0.01$)

b. **Gross pathology** No treatment related macroscopic changes were found.

c. **Microscopic pathology** (Table 5)

Non-neoplastic lesions At 100 ppm, mineralized particles were observed in the colloid of isolated follicles of 12 of 50 male thyroid glands ($p<0.01$) at 24 month sacrifice.

At 300 ppm these thyroid lesions occurred in 31 of 50 males ($p<0.001$) at 24 months.

At 900 ppm the thyroid lesions were observed in 10 of 10 males ($p<0.05$) at 12 month sacrifice. At 24 months the incidence of this lesion was observed in 44 of 50 males ($p<0.001$) and 27 of 50 females ($p<0.01$).

TABLE 5. INCIDENCE OF MINERALIZED PARTICLES IN THYROID COLLOID

Dose Level	Incidence			
	Male		Female	
	12 months N=50	24 months N=50	12 months N=10	24 months N=50
0	3	2	0	11
100	3	12**	0	6
300	6	31**	0	11
900	10*	44***	3	27**
0	5	12	2	3
1800	10*	46***	0	38***

* = (p<0.05), ** = (p<0.01), *** = (p<0.001)

At 1800 ppm the thyroid lesions were observed in 10 of 10 males ($p<0.05$) at 12 month sacrifice. At 24 months the incidence of this lesion was 46 of 50 males ($p<0.001$) and 38 of 50 females ($p<0.001$).

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:

* Retinal atrophy --44% increase ($p<0.05$) in females.
* Porphyrin accumulation in Harderian glands --65% increase ($p<0.05$) in females.
* Nephropathy-- marked reduction in incidence in males (65%, $p<0.001$) and females (92%, $p<0.01$).

Neoplastic Lesions No treatment-related increases were observed at any dose level.

D. DISCUSSION:

NTN 33893 caused significant decreases in body weight in females at 900 ppm, and in both sexes at 1800 ppm. Body weight gain was affected at the high dose level only, in females. **The NOEL for decreased body weight is 300 ppm.** These minimal effects on body weight gain in one sex only suggests that testing at a higher dose level is warranted. However, decreases in body weight gain in excess of 20% were observed in

both sexes at 2400 ppm in a subchronic study, as well as other effects (see Dose Selection, page 3). Since the high-dose level of 1800 ppm is 75% of the toxic dose level of 2400 ppm, it is concluded that testing has been adequate under the guidelines.

Urinalysis analyses demonstrated decreased protein concentration in the urine of 1800 ppm males. A decrease in nephropathy was also observed in this group. The significance of these observations is unclear.

Significant organ weight changes were observed in both sexes at 900 ppm and above. The decreases in absolute liver (male and male) and kidney (female) weights, and the increases in relative brain and heart (female) weights at 900 ppm are easily correlated with the decreased body weight also observed at this level. At 1800 ppm similar changes were observed in both sexes, involving more organs. **The NOEL for organ weight changes is 300 ppm.**

Statistically significant increases in microscopic lesions were observed at all dose levels. Retinal atrophy in females, porphyrin accumulation in female Harderian glands, reduced nephropathy in males and females, were observed at 1800 ppm. In addition, thyroid lesions (mineralized particles in follicular colloid) were observed at all dose levels in males in a dose-related manner, and at 900 ppm and above in females. While there was a statistically significant increase in the incidence of lesions at 100 ppm in males compared with its concurrent control, the increase was within control levels for concurrent female controls, and also controls from the 1800 ppm study. Since no other effects were noted at this level, this finding at 100 ppm is not considered to be biologically significant. **The NOEL for microscopic pathology is 100 ppm.** The LOEL is 300 ppm.

No neoplastic lesions were observed. These data demonstrate that NTN 33893 is not oncogenic in the rat.

These two studies, taken separately, would each be inadequate for regulatory purposes, because each study is deficient in dosing levels. However, when considered together, these studies provide adequate data, with demonstrated treatment-related toxicity, to satisfy the requirements for a chronic/oncogenicity study.

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Reviewed by: Myron S. Ottley, Ph.D. 9/17/92
Section IV, Tox. Branch (H7509C)
Secondary reviewer: Marion P. Copley, DVM, DABT
Section IV, Tox. Branch (H7509C) 9/17/92

009960

DATA EVALUATION REPORT

STUDY TYPE: **Chronic -- Dog** (83-1b)

TOX. CHEM NO: 497E
PC NUMBER: 129099
MRID NO.: 422730-02

TEST MATERIAL: **NTN 33893 Technical**

SYNONYMS: Imidacloprid (proposed)

STUDY NUMBER: 100015

SPONSOR: Bayer, AG

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

TITLE OF REPORT: 52-Week Oral Toxicity (Feeding) Study With NTN 33893 Technical in the Dog

AUTHOR(S): T.R. Allen, Th. Frei, H. Luetkemeier, O. Vogel, K. Biedermann, J. Wilson

REPORT ISSUED: October 19, 1989

CONCLUSION:

NOEL: 1250 ppm (41 mg/kg/d)
LOEL: 2500 ppm (72 mg/kg/d) Increased cytochrome P-450 levels in ♂ and ♀. Considered a threshold dose. 5000 ppm caused 50% mortality in range-finding study.

Classification: **Core Minimum.** Although dose levels were not high enough to demonstrate toxicity such as body weight loss, 2500 ppm is considered adequate, it is based on results from the range-finding study.

This study satisfies the guideline requirements (83-1) for a Chronic Dog study on NTN 33893 Technical and is acceptable for regulatory purposes.

A. MATERIALS: 009960

1. **Test compound** NTN 33893 Technical; Description: Solid, Batch #: 180587; Purity: 94.9%.

2. **Test animals** Species: Dog; Strain: Beagle, Age: 4 - 6 months; Weight: 6.6 - 9.2 kg (males), 5.3 - 7.4 kg (females); Source: Laboratory Research Enterprises, Inc., Kalamazoo, Michigan.

B. STUDY DESIGN:

1. **Animal assignment** Animals were fed NTN Technical in the diet daily for 52 weeks, and examined for signs of toxicity. They were assigned randomly as illustrated below.

Table 1.

Dose Group	Animals/Group		Conc. in Feed, ppm	Average Intake, mg/kg/d*
	Male	Female		
Control	4	4	0	0
Low Dose	4	4	200	6.1
Mid Dose	4	4	500	15
High Dose **	4	4	1250/2500	41/72

* Provided by authors
** Adjusted upward from Week 17 onwards

2. **Dose Selection** Dose levels were based on the results of a 4-week range-finding study (MRID #422563-30). Groups containing 2 male and 2 female beagle dogs were exposed to 0, 200, 1000 and 5000 ppm admixed in the diet for 28 days.

In the high-dose group, ataxia, tremors and occasional vomiting were observed. All animals in this group died or were killed in moribund condition. Significant reductions in food consumption and body weight were observed.

In the mid-dose group, no clinical signs were observed. Food consumption was reduced (described as slight to moderate) for the first part of the study only. Body weights were not affected.

In the low-dose group, no clinical signs were observed. Food consumption and body weights were not affected.

Other observations, such as clinical biochemistry, organ weights, macroscopic and microscopic findings, indicated no toxicity below the lethal dose (5000 ppm) with the exception of increased relative liver weight in one male at 1000 ppm and slight increases in cytochrome P-450 levels of 3 of the 4 animals at 1000 ppm.

In a 13-week feeding study (MRID 422563-28) groups of 4 male and 4 female beagle dogs were treated with NTN 33893 technical at 0, 200, 600, and 1800 ppm. The high dose was lowered to 1200 ppm from Week 4 due to low food consumption. No adverse effects were seen when all standard endpoints/assays were examined.

From these two studies, 1000 ppm was considered to be the minimally toxic level, and 1200 ppm appeared to be near the highest concentration tolerated in the feed. 1250 ppm was selected as the high dose level for the 52-week study. Absence of significant toxicity led to the increasing of the high-dose to 2500 ppm from week 17 onwards.

3. **Diet preparation** Diet was prepared every two weeks and stored at room temperature. Samples of treated food were analyzed for homogeneity, stability and concentration before test initiation and every three months thereafter.

4. **Food and Water Administration** Animals received 300±1 gm food daily between 10 a.m. and 1 p.m. Water was supplied *ad libitum*.

5. **Statistics** A copy of the statistical procedures is attached.

6. **Quality Assurance** A signed quality assurance statement was attached.

C. METHODS AND RESULTS

1. **Clinical Observations** Animals were inspected at least twice daily for signs of toxicity and mortality.

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

2. **Food consumption and compound intake** Consumption was determined and mean daily diet consumption was calculated. Compound intake were calculated from the consumption and body weight gain data.

   No changes in food consumption were observed in the low-dose or mid-dose groups. Although transient changes in food consumption were observed in the high dose group, as described in the next two paragraphs, these changes were not statistically significant.

   In high-dose males a 3% decrease in food consumption (g/animal/day) was observed

during Week 1, compared with pre-dose levels; in high-dose females a 15% decrease was observed during Weeks 1 & 2. Thereafter, in high-dose males and females, food consumption values were similar to controls.

In Week 17, high-dose concentrations in the diet were increased 100% from 1250 ppm to 2500 ppm. Again, there was a transient decrease of 5% in males during Weeks 17 and 18; in females a decrease of 9% was observed during Weeks 17 - 19, and a decrease of 5% was observed in Week 20.

3. **Body weight** Animals were weighed weekly and at terminal sacrifice.

No statistically significant dose-related changes in body weight or body weight gain were observed in any dose group. However, as seen in table 2, in males there was a trend towards decreased body weight gain as a function of dose.

TABLE 2. EFFECT OF TREATMENT ON PERCENT BODY WEIGHT GAIN

	Percent Gain in Group Mean Body Weight from Pretest to Week 52			
Dose Level ☞	0 ppm	200 ppm	500 ppm	1250/2500 ppm
Males	31	29	28	27
Females	34	38	28	32

4. **Hearing Tests** Each animal was tested for hearing impairment using a simple noise test on weeks 13, 26 and 52 of the study.

No effects on hearing ability were observed.

5. **Ophthalmological examination** Each animal was tested for abnormalities of the eyes, using the Heine-Bifocal ophthalmoscope approximateiy 20 min. after the instillation of 0.5% tropicamide solution. Examinations were performed during weeks 13, 26 and 52.

No effects on ophthalmological parameters were observed.

6. **Blood** was collected from all animals before treatment and during weeks 13, 26 and 52 for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. **Hematology**

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

No effects on hematological parameters were observed.

b. **Clinical Chemistry**

X		X	
	Electrolytes:		**Other:**
X	Calcium		Albumin
X	Chloride	X	Blood creatinine
	Magnesium		Blood urea nitrogen
X	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)	X	Triglycerides
X	Creatinine phosphokinase	X	Serum protein electrophores
X	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

Plasma Cholesterol levels were 56% higher in the high-dose females ($p < 0.01$) during Week 13 and 62% ($p \leq 0.05$) during Week 26. The 67% increase noted during Week 52 was not statistically significant.

Hepatic **cytochrome P-450** levels, measured at the end of the study period, were 94% above controls ($p \leq 0.01$) in high-dose males, and 51% above controls in high-dose females. P-450 levels were not measured at lower dose levels.

No other toxicologically significant changes in clinical chemistry parameters were observed.

6. **Urinalysis** Urine was collected from fasted animals at the start of the study, and at weeks 13, 26 and 52. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
	Volume	X	Ketones
X	Specific gravity	X	Bilirubin
X	Ph	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

No effects on urinalysis parameters were obse ved.

7. **Sacrifice and Pathology**

Dogs were anaesthetized by i.v. injection of Narcoren, and then killed by exsanguination. All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

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

a. **Organ weights** No significant changes in organ weights were observed.

b. **Gross pathology** No treatment related macroscopic changes were found.

c. **Microscopic pathology** No treatment related microscopic changes were found.

D. **DISCUSSION:**

The gross toxic manifestations observed following NTN 33893 administration in the diet for 52 weeks were transient decreases in food consumption in both sexes during

Week 1 (males) and Weeks 1 and 2 (females). Similarly, when the high dose level was doubled in Week 17, males showed a transient decrease in food consumption during Weeks 17 and 18, and in females during Weeks 17 - 20. Although the NOEL for decreased food consumption was 500 ppm, the mid-dose, it is probably due to palatability and will not be used for regulatory purposes.

Clinical biochemistry assays showed effects at 2500 ppm (only controls and high dose level tested): increases in plasma cholesterol in females, and increases in liver cytochrome P-450 levels in both sexes. The elevated plasma cholesterol levels were also observed at Week 13 when the high dose level was 1250 ppm.

However, no overt toxic effects were observed at 1250 ppm or 2500 ppm. Neither the elevated plasma cholesterol levels observed in females, nor the elevated P-450 levels in both sexes, could be correlated with other toxic manifestations. Therefore, **1250 ppm is considered the NOEL.** It would appear that 2500 ppm is at or near the threshold of toxicity, but because the animals were dosed at 2500 ppm only from Week 17 onward (69% of the 52-wk period), the degree of toxicity observed at this level is expected to be diminished compared with a full 52-week dosing period. And since hepatic cytochrome P-450 levels were definitely elevated at this level, **2500 ppm should be considered a LOEL.**

It would be useful to have data on P-450 levels at doses lower than 2500 ppm, in order to better shape the profile of toxicity of NTN. However, since elevated P-450 levels at 2500 ppm could not be correlated with other toxic manifestations, absence of data at the lower doses does not change the conclusions of this study.

Dose levels were based on a rangefinding study, in which 1,800 ppm resulted in severely reduced food intake (presumably because of unpalatability), and 5,000 ppm produced significant (50%) death. Since 2500 ppm is 50% of this lethal dose, the results of this study are considered acceptable for regulatory purposes.

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[illegible] Tox Branch I (H7509C)

Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T.

[illegible] Tox Branch I (H7509C) Marion Copley 1/8/93

DATA EVALUATION REPORT

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) 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 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

Maternal

NOEL =24 mg/kg/d

LOEL =72 mg/kg/d: decreased body weight, increased resorption, increased abortion, and death)

Developmental

NOEL = 24 mg/kg/d

LOEL = 72 mg/kg/d (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.

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 9/25/87 (42 days after last sacrifice).

2. **Test Animals:** Species & Strain: Rabbit, Chinchilla (CHbb: CH Hybrids, SPF Quality); Age: 4 - 6 weeks at pairing; Weight at day 0 post coitum: 2650 - 4064 gm; Source: KFM, Kleintierfarm Madoerin AG, Switzerland.

B. STUDY DESIGN

1. **Animal Assignment** Four groups of 16 dams were exposed to NTN 33893 Technical orally by gavage from days six through 18 post coitum as follows:

Group 1	0 mg/kg body weight/day (vehicle control)
Group 2	8 mg/kg body weight/day
Group 3	24 mg/kg body weight/day
Group 4	72 mg/kg body weight/day

2. **Dose Selection** was reportedly based on the results of a range finding study (RCC Project 083520). data were not submitted, nor were details provided. Dose volume remained a constant 4 ml/kg, and concentrations were adjusted daily to correspond with body weight. The vehicle was distilled water with 0.5% Cremophor EL (BASF).

3. **Food and Water** Standard Kliba 341 rabbit maintenance diet was available ad libitum. Tap water was also available ad libitum through an automatic watering system.

4. **Housing/Environmental Conditions** Animals were housed individually in stainless steel cages equipped with an automatic cleaning system. Temperature range was 22 ± 3°C; Humidity range was 40% - 70%. Light/dark cycle was 12hr/12hr, with low-volume music played for at least eight hours 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 copulation had been observed, and individually thereafter. The day of mating was considered Gestational Day 0 (gd 0).

   Sixteen 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.

   In the high-dose group (72 mg/kg/d) two females died during the study, one each on days 18 and 19. One female excreted white mucoid feces during the three days preceding death. These females both carried litters, the sizes of which corresponded with corpora lutea counts. A third high-dose female aborted her litter on gd 26, while two others showed evidence of total resorption. These effects were considered treatment-related. No other treatment-related signs of toxicity were noted a the high-dose level, or in the lower dose levels.

3. **Food Consumption** was recorded on gd 6, 11, 15, 19, 24, and 29.

As seen in Table 1, in the high-dose group, food consumption decreased ($p \leq 0.01$) relative to controls during gd 6 through 19, which was the treatment period. During the last five days of gestation, food consumption was increased compared with controls ($p \leq 0.05$).

In the mid-dose group, food consumption was 22.5% lower than controls ($p \leq 0.05$) during gd 6 - 11 only, when compared on a gram/animal basis. The authors did not report analysis of g/kg/day food consumption comparisons with controls, but examination of the data show that food consumption in the mid-dose group was only 9.9% less than controls during this period. Since this decrease is transient and it is the only effect at this level the mid dose will be considered the NOEL.

TABLE 1. MEAN FOOD CONSUMPTION AS IT DIFFERED FROM CONTROLS (G/ANIMAL/DAY)

Gestational Day		Control	Mid-Dose 24 mg/kg	High-Dose 72 mg/kg
0 - 6	Mean ± S.D. N	207.3 ± 27.4 13	214.3 ± 17.2 16	206.1 ± 26.5 8
6 - 11	Mean ± S.D. N	198.7 ± 35.0 13	166.1* ± 27.9 14	66.9** ± 16.3 7
11 - 15	Mean ± S.D. N	194.3 ± 24.3 13	190.6 ± 27.0 13	112.1** ±49.3 6
15 - 19	Mean ± S.D. N	197.5 ± 31.2 12	206.1 ± 42.4 12	87.1** ± 64.7 4
19 - 24	Mean ± S.D. N	160.0 ± 47.3 10	196.0 ± 24.7 10	178.9 ± 42.9 3
24 - 28	Mean ± S.D. N	108.3 ± 42.6 10	132.8 ± 32.1 7	198.2* ± 52.0 3

4. **Body Weights** were recorded daily from gd 0 through 28.

As seen in Table 2, mean **body weights** were lower than controls in the high-dose groups during much of the treatment period. Recovery was observed following treatment. Body weights in the lower dose groups were not significantly different from controls.

TABLE 2. CHANGE IN BODY WEIGHT IN THE HIGH-DOSE GROUP, COMPARED WITH CONTROLS

Gestational Day(s)	Range of Body Weight Change Compared with Controls (%)	P Value (Dunnett Test)
11 - 16	-8 to -9	($p \leq 0.05$)
17 - 21	-10 to -11	($p \leq 0.01$)
22, 23, 25, 26	-10 to - 8	($p \leq 0.05$)

Body weight gain in the low-dose group (8 mg/kg/d) was similar to the controls. In the mid-dose and high-dose groups, the profile of body weight gain correlated with changes in food consumption. Maximum difference in the high-dose vs. the controls was 9.2%. When corrected body weight gain was examined, the difference between control and high dose groups was -2.3%. None of these changes was statistically significant.

5. **Terminal Sacrifice and Post Mortem Examination** Animals were killed by cervical dislocation on gd 28 and fetuses were removed by Caesarean section. Females internal organs were examined macroscopically. Uterine contents, position of fetuses in the uterus and number of corpora lutea were noted and recorded. 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** (See attached)

As mentioned earlier, one high-dose female aborted two days prior to parturition. Two other high-dose females had total resorptions. Assessing only females with live fetuses at necropsy, there was a 10.8% ($p \leq 0.05$) increase in post-implantation loss in the high-dose group. When the females with total litter loss are included in the analysis, post-implantation loss in the high dose group is 32.5% ($p \leq 0.01$), with a corresponding 31% decrease (not statistically significant due to high S.D.) in number of live fetuses per litter.

b. **Fetal Data**

Mean **Litter Weight** was 9.7% lower ($p \leq 0.05$) in the high dose group compared with controls. Mean **fetal weight** was also lower (9.9%, $p \leq 0.01$) than controls at this dose level. Similar reductions in litter and fetal weights were noted at the low-dose level (8 mg/kg/d); however, these

appeared to be caused by one female with abnormally low litter and fetal weights. And since mid-dose weights were similar to controls, the authors did not consider this low-dose observation to be treatment-related.

External and Visceral observations revealed no treatment-related effects apart from the presence of three runts fetuses from two litters.

Skeletal Abnormalities were observed in five fetuses (6%) from three high-dose females, compared with two fetuses from one female in the controls. The nature of the malformations was not different between the two groups. Specific malformations are listed in Table 3. Statistical significance was not reported.

TABLE 3. INCIDENCE OF SKELETAL ABNORMALITIES IN HIGH-DOSE GROUP

Nature of Abnormalities	Controls 136 fetuses in 16 litters		High Dose Group 83 fetuses in 11 litters	
	Incidence in Fetuses**	Incidence in Litters	Incidence in Fetuses**	Incidence in Litters
Partially fused ribs	1	1	0	0
Misshaped thoracic vertebral body #4	1	1	0	0
Sternebrae fused	0	0	2	2
Sternebrae asymmetric	3	0	3	2
Sternebrae missing	0	0	2	1
Sternebrae abnormally ossified	0	0	4	2
Shortened tail	0	0	1	1

** Number of fetuses examined = 83. Some fetuses had more than one malformation

D. SUMMARY

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Table 4 summarizes the levels at which significant adverse effects were observed in female rabbits and their offspring.

TABLE 4. SUMMARY OF ADVERSE EFFECTS

Endpoint Adversely Effected	LOEL, mg kg/d	NOEL, mg/kg/day
Clinical Observations Mortality	72	24
Food Consumption	72	24
Body Weights	72	24
Female Reproduction	72	24
Fetal Weight	72	24
Skeletal Abnormalities	72	24

NTN 33893 caused adverse effects in the rabbit conceptus only at dose levels that produced significant maternal effects, including death. 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 artifactual to maternal effects.

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