


CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY  
DEPARTMENT OF PESTICIDE REGULATION  
HUMAN HEALTH ASSESSMENT BRANCH

**Summary of Toxicological Data: Imidacloprid**

Chemical Code: 3849, Document Processing Number (DPN): 51950

Original: 5/24/93

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## INTRODUCTION

### DPR Background

**File Name:** T240126

**DPR Chemical Code:** 3849

**Data Volume Nos.:** 51950-0002 to -0008, -0010, -0011, -0013 to -0021, -0024 to -0026, -0114 to -0116, -0158, -0210, -0255, -0258, -0279, -0471 to -0474, -0684 to -0689, -0835, -0998

**SB 950:** N/A

**Original:** T040330, 3/30/04

**Revised:** T240126, 1/26/24

**About This Document:** Toxicology one-liners are attached. All record numbers for the above study types W119443 through W344610, (Data Volume Numbers 51950-0002 through 51950-0998) were examined. This includes all relevant studies indexed by DPR as of 1/26/24. This record contains summaries of studies. Individual worksheets may be useful for detailed assessment. In the 1-liners below: \*\* indicates an acceptable study, and † indicates a potential health effect was identified.

### Chemical Properties

**IUPAC Name:** (NE)-N-[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]nitramide

**Synonyms:** 1-((6-chloro-3-pyridinyl)methyl)-N-nitro-2-imidazolidinimine

**CAS No.:** [138261-41-3](#)

**Description:** white to beige powder

**Molecular Weight:** 255.7

**Melting Point:** 144° C

**Boiling Point:** 442.3° C at 760 mm Hg

**Density:** 1.54 g/ml

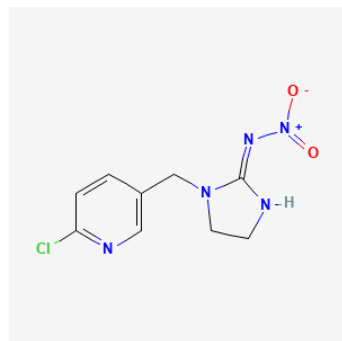
**Dissociation Constant:** pka1: 1.56, pka2: 11.12

**Partition Coefficient:** log  $k_{ow}$ : 0.57

**Water Solubility:** 610 mg/l

**Vapor Pressure:**  $3 \times 10^{-12}$  mm Hg at 20° C

### Chemical Structure



[Imidacloprid | C9H10ClN5O2 - PubChem \(nih.gov\)](#)

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## HAZARD IDENTIFICATION SUMMARY

Imidacloprid is a systemic insecticide belonging to the neonicotinoid chemical class. It acts to block neural transmission in nicotinic pathways. In the ADME evaluations, the chemical was readily absorbed via the gastrointestinal tract and quickly excreted by renal pathway in the urine. The chemical did not significantly penetrate the blood/brain barrier in the tissue distribution assessment. Minimal bioaccumulation was noted in the multiple dosing regimen.

The predominant acute toxic hazard was via the oral route. The hazard was Category II with clinical signs of tremors and other signs of neurotoxicity. The acute neurotoxicity study results confirmed the neurotoxic effects of imidacloprid. The other acute toxicity hazards were Category IV with no indication of a dermal sensitization potential.

Longer-term treatment indicated the liver to be the target organ. Liver necrosis was noted in the rat subchronic toxicity study. No effect levels (NOEL) of 15 and 5 mg/kg/day were noted in the rat subchronic and chronic toxicity studies, respectively. The rare liver tumor, cholangiocellular carcinoma, was identified in the rat combined chronic and oncogenicity study. Effects on the liver were also observed in the dog chronic and mouse oncogenicity studies with NOELs of 12.5 and 143 mg/kg/day being noted. However oncogenic effects were not evident in these studies. In the genotoxicity battery, *in vitro* chromosomal aberrations were evident in two studies which may be indicative of an oncogenic potential. Otherwise no positive responses were noted in the other submitted genotoxicity studies. The subchronic neurotoxicity study results did not manifest signs of toxicity. The lack of a neurotoxic effect was likely due to the extended time of exposure resulting from a dietary uptake versus the bolus effect of the acute oral gavage dosing. In the more recent developmental neurotoxicity study, a more detailed evaluation of the pups identified a treatment-related effect at a LOEL of 17.9 mg/kg/day (NOEL: 7.4 mg/kg/day).

No toxicologically significant effects were identified in the rat two-generation reproduction and rat and rabbit developmental toxicity studies with NOELs in the range of 20 to 25 mg/kg/day. In regard to immunological competency, a possible treatment-related effect of diminished delayed type hypersensitivity response and T-cell stimulation was noted in a reviewed literature study.

## METABOLISM AND PHARMACOKINETICS

NOTE: DPR has determined that the following series of studies, each considered supplementary, fulfills the requirements for metabolism and pharmacokinetics studies.

51950-0021 119510; "Methylene-[<sup>14</sup>C] Imidacloprid: Metabolism Part of the General Metabolism Study in Rat" (Authors: Klein, O. and Karl, W., Bayer AG, Leverkusen-Bayerwerk, Germany, Report # 101999, 1/30/90); 851; rat; A: NTN 33893, 99.9% purity), B: 1-[(6-chloro-3-pyridinyl)<sup>14</sup>C-methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine] (150.7 μCi/mg, >99% purity); oral: single (1 mg/kg B, 20 mg/kg B), multiple (1 mg/kg A qd @ 14 days followed by 24 hrs after final dose by a single dose of B); IV: single (1 mg/kg B); 5 rats/sex/dose; >90% of administered radioactivity eliminated 48 hours after dosing with less than 1% remaining in the carcass in all dose groups; no sex differences in excretion pattern and

metabolic profiles of the excreta were evident following low dose administration; however, at the high dose, females showed a slightly higher renal elimination rate than males; males showed a higher capacity to metabolize the test compound and the amount of parent compound was lower as compared to females; oxidation cleavage of the parent compound yields 6-chloronicotinic acid which then reacts with glycine to form a conjugate (WAK 3583); second major route of metabolism involves hydroxylation and elimination of water from the imidazolidine ring in the 4- or 5-position to produce the metabolite NTN 35884; these metabolites are excreted in urine and feces; no evidence of bioaccumulation following multiple dosing was reported; supplemental; (██████████ 4/14/93).

51950-0021 119511; “[<sup>14</sup>C]-NTN 33893: Investigations on the Distribution of the Total Radioactivity in the Rat by Whole-Body Autoradiography” (Author: Klein, O., Bayer AG, Leverkusen-Bayerwerk, Germany, Report # 87264, 11/20/87); 851; 1-[(6-chloro-3-pyridinyl)<sup>14</sup>C- methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine] (150.7 μCi/mg, >99% purity); oral: 20 mg/kg to 6 male Wistar BOR:WISW (SPF Cpb) rats and killed at 1, 4, 8, 24, and 48 hrs after dosing; IV: 20 mg/kg to 1 rat and killed 5 minutes after dosing; with the exception of fatty tissues, the central nervous system, and the mineral part of the bones, radioactivity over all parts of the body was observed 5 minutes after IV injection as well as 1 hour following oral dosage; in addition, high amounts of radioactivity in the liver were reported at this time; after 24 hours, high level of radioactivity in the kidney indicates high rate of renal excretion of the administered radioactivity; concentrations of radioactivity in fatty tissues and central nervous system were very low during the whole period of study suggesting that the parent compound and its metabolites are not very lipophilic and do not readily pass through the blood brain barrier; with increasing time after administration, the concentration of radioactivity decreases in most organs and tissues; relatively high blackening over the nasal mucosa as compared to background observed at the end of the study indicates that the administered radioactivity is not only excreted via urine and feces, but might also be excreted to a small extent with the nasal mucus; supplemental; (██████████,4/14/93).

51950-0021 119512; “[<sup>14</sup>C]-NTN 33893: Biokinetic part of the 'General Metabolism Study' in the Rat” (Author: Klein, O., Bayer AG, Leverkusen-Bayerwerk, Germany, Report # 87265, 9/30/87); 851; rat; A: NTN 33893, 99.9% purity), B: 1-[(6-chloro-3-pyridinyl) <sup>14</sup>C-methyl]-4,5-dihydro- N-nitro-1H-imidazol-2-amine] (150.7 μCi/mg, >99% purity); oral: single (1 mg/kg B, 20 mg/kg B), multiple (1 mg/kg daily for 14 days followed by 24 hrs after final dose by a single dose of B); IV: single (1 mg/kg B); 5 rats/sex/dose; following oral and intravenous administration 94 - 100% of the administered radioactivity is absorbed and readily distributed to the body from the central compartment as indicated by a short mean absorption half-life (35 minutes) and an apparent volume of distribution accounting for about 84% of the total body volume; the small mean residence time which vary between 9 and 17 hours suggests that the total radioactivity is rapidly eliminated from the body; after oral or intravenous administration, 91.4 to 96% of the given dose was excreted via urine and feces by 48 hours; no significant amount of radioactivity was found in expired air; high concentrations of total radioactivity were observed in the kidney, liver, lung and skin; no signs of bioaccumulation were evident; supplemental; (██████████ 4/15/93).

51950-0021 119513; “[Imidazolidine-4,5-<sup>14</sup>C] Imidacloprid: Investigation of the Biokinetic Behavior and Metabolism in the Rat” (Authors: Klein, O. and Brauner, A., Bayer AG, Leverkusen-Bayerwerk, Germany, Report # 102617, 1/11/91); 851; rat; [Imidazolidine-4,5-<sup>14</sup>C] Imidacloprid (0.827 μCi/mg, 99.8% purity, Batch # 890315ELB01 and 124 μCi/mg, >99% purity, Batch # not reported); oral; 1 mg/kg (10 males, 5 females) and 150 mg/kg (5 males); absorption after oral dosing is rapid and maximal plasma concentration is achieved between 1 and 1.5 hr at the low dose and 4 hrs at the high dose; after oral

administration of the imidazolidine labeled compound, the renal-excreted portion of the given dose is higher (91%) as compared to methylene labeled Imidacloprid (75%); fecal elimination plays a minor role and 1% of the administered radioactivity remains in the body at 48 hrs; highest radioactivity concentrations were reported in liver irrespective of dose level; 5 metabolites were identified in urine which represent 77% of radioactivity recovered in urine; supplemental; (██████), 4/15/93).

51950-0026 119532; "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" (Author: Klein, O., Bayer AG, Leverkusen-Bayerwerk, Germany, Report # 100645, 7/17/90); 851; methylene-[<sup>14</sup>C] imidacloprid (86.4 - 123 μCi/mg, 98.4 - 99% purity): single [1 mg/kg (5 males), 150 mg/kg (7 males)] and chronic treatment with the unlabeled imidacloprid for 1 year in diet prior to receiving radiolabeled imidacloprid (80 mg/kg, 10 males); methylene-[<sup>14</sup>C] WAK 3839 (40 μCi/mg, 99% purity): 1 mg/kg 5 males); both compound absorbed rapidly after single oral dosing; terminal half-lives for imidacloprid and WAK 3839 are 35.7 and 46.9 hrs, respectively; 75% of the given dose of both compounds are eliminated primarily via urine within 48 hrs; fecal elimination plays a minor role, since 21% and 16% of the recovered radioactivity are excreted by this route, respectively; glycine conjugate of 6-Cl-nicotinic acid (WAK 3583), two monohydroxylated metabolites (WAK 4103) and the unsaturated metabolite (NTN 35884) were identified in the urine and accounted for 82% of the total radioactivity; same metabolites were also identified in feces; besides unchanged WAK 3839, one other metabolite NTN 33823 was identified in urine and feces obtained from rats treated with WAK 3839; WAK 3839 and other metabolites identified after a single low dose were detected in urine from rats and mice treated chronically with imidacloprid in their diet; this finding suggests that WAK 3839 is formed during chronic exposure to imidacloprid; supplemental; (██████) 4/16/93).

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## GUIDELINE ACUTE STUDIES ON ACTIVE INGREDIENT

### Acute Oral Toxicity, Rat

\*\*002; 119443; "NTN 33893 Study for Acute Oral Toxicity to Rats" (Author: Bomann, W., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 100040, 12/15/89); 811; NTN 33893 techn. (94.2% a.i.); 5/sex/dose; single doses of 50 (males only), 100, 250, 315, 400, 450, 475 (females only), 500, 1800 mg/kg; mortalities- males: 0/5, 0/5, 0/5, 0/5, 1/5, 4/5, 5/5, 5/5, respectively; females: 0/5, 0/5, 0/5, 1/5, 0/5, 5/5, 5/5, 5/5, respectively; observations- no clinical signs in male 50 mg/kg dose group or in female 100 mg/kg dose group; apathy and labored breathing in all male test animals in the 100 mg/kg dose group, signs clearing in all within 1 day; apathy, decreased motility, narrowed palpebral fissure, tremors, staggering gait, accelerated and/or labored breathing, and spasmodic state in the other dose groups, all signs clearing in the survivors within 6 days; necropsy- mortalities: dark livers, pale spleens, and dark, patchy and distended lungs, and, in 3/5 females in the 475 mg/kg group, glandular stomach reddened; survivors: no test substance-related changes; approximate LD 50 (M)= 424 mg/kg; estimated LD 50 (F)> 450 mg/kg and <475 mg/kg; NOEL (M/F)=50 mg/kg; Category II; Acceptable (██████) 4/6/93)

\*\*003 119462; "NTN 33893 Study For Acute Oral Toxicity To Mice" (Author: Bomann, W., Bayer AG Department Of Toxicology, Wuppertal, West Germany; Report # 100039; 12/15/89); 811; NTN 33893 technical (Purity = 94.2%) mixed with 2% v/v Cremophor EL in demineralized water; oral doses of (M) 10, 71, 100, 120, 140, 160, 250 and (F) 10, 100, 120, 140, 160, 250 mg/kg; 5 mice/dose; mortalities (M)

0/5, 0/5, 1/5, 2/5, 2/5, 5/5, 5/5 and (F) 0/5, 0/5, 1/5, 1/5, 2/5 and 5/5, respectively; clinical observations included apathy, labored breathing, decreased motility, staggering gait, trembling and spasms; all symptoms cleared within 7 hours of dosing; necropsy revealed liver pale - occasionally dark, spleen pale - occasionally dark, lungs dark patchy and distended; LD50 = (M) 131 mg/kg and (F) 168 mg/kg; NOEL = 10 mg/kg; Toxicity Category II; study acceptable. (██████████ 4/6/93)

\*\*51950-0684; 237737; Acute Oral Toxicity; 811; rat; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5702; 03/04/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w imidacloprid; 260, 380, and 550 mg/kg; single, oral-gavage dose, with a 14-day observation period; 2, 4, or 6 female test subjects/dose level; mortality: none; clinical signs: 260, none reported; 380, tremors (5/6) and lethargy (6/6) and abdominal breathing (1/6); and 550, tremors (2/2) and lethargy (1/2); body-weight gain was positive in all survivors throughout; necropsy: congested lung lobes in all (2/6) or some (1/6), congested brain (4/6), and/or mottled liver (1/6); 260, no gross abnormalities reported; 380, congested brain (4/6), congestion of at least one (1/6) or all lung lobes (2/6), and/or mottling of liver (1/6); 550, congestion of all lung lobes (2/2), congested brain (2/2), and/or mottled liver (1/2); LD 50 (F) = 380 (296 - 428) mg/kg; Toxicity Category II; Acceptable. (██████████ 05/14/08)

#### **Acute Dermal Toxicity, Rat**

\*\*002; 119446; "NTN 33893 (c.n. Imidacloprid (Proposed)), Study for Acute Dermal Toxicity to Rats" (Author: Krötlinger, F., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 100041, 11/15/89); 812; NTN 33893 techn. (94.2% a.i.); 5/sex/dose; 1 dose (moistened with 0.9% NaCl solution) of 5000 mg/kg; 24 hr exposure, covered; no mortalities; observations- no clinical signs; necropsy- no treatment related lesions; LD 50 (M/F) > 5000 mg/kg; Category IV; Acceptable. (██████████, 4/7/93)

\*\*51950-0685; 237738; Acute Dermal Toxicity; 812; rat; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5703; 02/20/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w imidacloprid; 0 (5 F) and 5.0 g/kg (5 M/5F); single, 24-hour, dermal exposure, with a semi-occlusive wrap and a 14-day observation period; 5 test subjects/sex/treatment level; mortality: none; clinical signs: none reported; body-weight gain was positive in throughout; necropsy: (0.0 g/kg) consolidation of right middle lung lobe (1/5 F); (5.0 g/kg) granular spleen (1/5 F); LD 50 (M/F) > 5.0 g/kg; Toxicity Category IV; Acceptable. (██████████ 05/15/08)

#### **Acute Inhalation Toxicity, Rat**

\*\*51950-002; 119449; "NTN 33893 Study for Acute Inhalation Toxicity in the Rat In Accordance with OECD Guideline No. 403, Data Requirement, EPA Guideline No. 81-3" (Author: Pauluhn, J., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 99806, 6/6/88); 813; NTN 33893 (technical agent, 95.3% a.i.); 5/sex/dose; doses (mean gravimetric concentration) of 1.220, 2.577, 5.323 mg/l; MMAD determinations of 10.6, 14.6, 20.0 m, respectively with GSD of 1.82, 1.92, 2.15, respectively; 4 hr exposure (head-nose only); no mortalities; observations- no clinical signs in the 1.220 mg/l dose group; difficult breathing, reduced motility, and piloerection in the 2.566 and 5.323 mg/l dose groups, and slight tremors in the 5.323 mg/l dose group after exposure, all signs clearing within 6 hrs; necropsy- no pathologic findings in the 1.220 mg/l group, isolated bilious foci in lungs of 4/5 males in the 2.566 mg/l group, and hepatic foci in lungs of 2/5 males in the 5.323 mg/l group; LC 50 (M/F) > 5.323 mg/l; Category IV; Acceptable. (██████████, 4/8/93)

\*\*51950-0686; 237739; Acute Inhalation Toxicity; 813; rat; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5707; 03/21/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w imidacloprid; gravimetric concentration: 0.0 and 0.589 mg/L; nominal concentration: not specified; average MMAD (GSD): 1.77 (2.54) m; single, 4-hour, head/nose-only, inhalation exposure, with a 14-day observation period; (0.0 mg/L) 5 female subjects/exposure level, (0.589 mg/L) 5 test subjects/sex/exposure level; mortality: none; clinical signs: (in-chamber) none reported; (post-exposure) none reported; body-weight gain was positive in all survivors throughout; necropsy: no gross abnormalities reported; reported LC 50 (M/F) > 0.589 mg/L; Toxicity Category III; Acceptable. (██████████ 05/16/08)

### Primary Eye Irritation, Rabbit

\*\*002; 119452; "NTN 33893, Study for Irritant/Corrosive Potential on the Eye (Rabbit) According to OECD Guideline No. 405" (Author: Pauluhn, J., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 99679, 2/25/88); OECD Guideline No. 405; NTN 33893 (technical agent, 94.2% a.i.); 3 animals; 0.1 ml volume (approximately 60 mg)/treated eye; observations- no corneal opacity or iritis; conjunctival irritation (grade 2 in 1/3, grade 1 in 2/3) 1 hr after test article instillation, clearing in all within 24 hrs; Category IV; Acceptable. (██████████ 4/12/93)

\*\*51950-0687; 237740; Primary Eye Irritation; 814; rabbit; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5705; 02/14/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w imidacloprid; 0.1 g/eye (neat); single, ocular instillation, with a 72-hour observation period; 3 male test subjects/treatment level; mortality: none; clinical signs: no signs of systemic toxicity were reported; corneal opacity, none reported; iritis, none reported; conjunctivae, redness - none reported; chemosis - none reported; discharge - none reported; reported Maximum Mean Total Score = 0.0; Toxicity Category IV; Acceptable. (██████████, 05/16/08)

### Primary Dermal Irritation, Rabbit

\*\*002; 119455; "NTN 33893, Study for Irritant/Corrosive Potential on the Skin (Rabbit) According to OECD Guideline No. 404" (Author: Pauluhn, J., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 99804, 2/25/88); OECD Guideline No. 404; NTN 33893 (technical agent, 94.2% a.i.); 3 animals; 500 mg (mixed to a paste with water)/animal; 4 hr exposure, covered; observations- no edema; grade 1 erythema in 1/6 1 hr after patch removal, clearing within 24 hrs; Category IV; Acceptable. (██████████ 4/12/93)

\*\*51950-0688; 237741; Primary Dermal Irritation; 815; rabbit; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5704; 02/20/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w imidacloprid; 0.5 g/site (moistened with 0.5 mL distilled water); single, 4-hour, dermal exposure, with a semi-occlusive wrap and a 72-hour observation period; 3 female test subject(s)/treatment level; mortality: none; clinical signs: no systemic toxicity reported; erythema, none reported; edema, none reported; reported Primary Dermal Irritation Index (PDII) = 0.0; Toxicity Category IV; **Acceptable.** (██████████, 05/19/08)

### Dermal Sensitization, Mouse

This product is not a dermal sensitizer (see WH&S memo, 06-13-93, Document #: 51950-002) according to Worker Health and Safety Branch Data Package Recommendation Sheet, July 19, 1993. Two studies were reviewed, i.e., Record #: 119458 (evaluating NTN 33893 technical imidacloprid in the



guinea pig maximization test) and 119459 (evaluating BAY NTN 33893 240 F. S. at 23.1% imidacloprid using the Buehler Topical Closed-Patch Technique).

\*\*51950-0689; 237742; Skin Sensitization; 816; guinea pig; Jai Research Foundation, Department of Chemistry, District Valsad, Gujarat, India; JRF Study #: 5706; 03/29/06; Imidacloprid Technical; Batch #: UPI-05/ID-208/27; composition: a. i., 98.1% w/w Imidacloprid technical; Guinea Pig Maximization Test; induction (10 test subjects/sex/treatment level): intradermal injections, 3 injections of 0.1 mL on each side of the spinal column of: (1) 1:1 (v/v) Freund's complete adjuvant (FCA with distilled water, (2) 2.5% (w/v) test article in propylene glycol; (3) 2.5% test article (w/v; in propylene glycol) in 1:1 mixture (v/v) FCA with distilled water; topical treatments, ~33% test-article (80% ethanol) for the induction treatments (Day 7) was applied following intradermal injections; challenge: 0.1 g test-article (moistened in 0.2 mL acetone) was applied, 21 days after (Day 21) the first intradermal injection, to the right flank of each subject; naïve-challenge: treatment conducted like those of the test group but subjects treated with 80% ethanol for the induction treatments, while the challenge treatments consisted of 0.1 g test-article (moistened in 0.2 mL acetone) to 5 test subjects/sex, placed on the right flank of each subject; positive control: 2-mercaptobenzothiazole (unspecified concentration and solvent) was used as the positive control (method not reported in detail); mortality: none; clinical signs: no systemic effects reported; induction treatments: (Day1) intradermal: (erythema) grade 2 in 7/20 (grade 1 in 13/20); (edema) grade 1 in 12/20; (Day10) topical: (erythema) grade 2 in 6/20 (grade 1 in 14/20); (edema) grade 1 in 7/20; challenge, (erythema) none reported at 24 or 48 hours; naïve-challenge, intradermal: (erythema) none reported; topical: (erythema) none reported; challenge, (erythema) none reported; body-weight gain was positive in all; test article was negative for dermal sensitization; Acceptable. (██████████ 05/20/08)

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## SUBCHRONIC STUDIES

### Ray Subchronic Dietary Toxicity

51950-0005 119467; "Subchronic Toxicity Study on Wistar Rats (Administration in the Feed for 96 Days)" (Author: R. Eiben; Bayer AG, Department of Toxicology, Wuppertal 1, West Germany; Report No. 100036; 7/14/89); NTN 33893 (Batch No. 180587; 95.3% imidacloprid); 0, 150, 600, and 2400 ppm in diet; 10 Wistar SPF rats/sex/dose level, plus 10/sex/dose level at 0 and 2400 ppm in a 4-wk recovery group; no mortality or clinical signs of toxicity; increased food consumption and decreased body weight at high-dose; decreased clotting time; possible adverse effect: liver necrosis (single-cell and foci) in high-dose males, occurred with cytoplasmic changes, swollen nuclei, increased serum AP and ALT, and decreased protein, albumin, triglycerides, and cholesterol; cytoplasmic changes were also observed in mid-dose males; NOEL = 150 ppm (based on liver effects in males); Acceptable. (██████████, 4/12/93)

### Rat 28-Day Inhalation Toxicity

\*\*51950-004 119465 Pauluhn, J., "Subacute Inhalation Toxicity Study on the Rat According to OECD Guideline No. 412," Bayer AG, Wuppertal, West Germany, 7/18/89. Laboratory Study No. 100262. NTN 33893 (imidacloprid, Batch No. 180 587; 95.2% purity), generated as a dust; 0 (air), 5.5, 30.5, 191.2 mg/m<sup>3</sup>/day (gravimetric); 10 Wistar SPF rats/sex/dose group; animals were exposed head-only for 6 hrs/day, 5 days/wk for 4 wks; MMADs (GSD) = 2.37 (1.5), 4.77 (2.0), and 5.70 (1.9) μm, respectively; no adverse effects; no mortality or clinical signs of toxicity; reduced body weights in high-dose males; liver effects including elevated serum enzymes and induction of hepatic MFOs in high-dose males and

females and mid-dose females; relative liver weight was increased and relative thymus weight was decreased in high-dose females; no toxicologically significant histological changes were observed; NOEL = 5.5 mg/m<sup>3</sup>/day (based on reversible changes in serum and hepatic enzymes). Originally designated as Supplemental (██████████ and ██████████ 4/7/93). These reviewers found the study to be valid, but not applicable to contemporary guidelines due to 4-week duration. More recently, U.S. EPA has made provision for 4-week inhalation studies to address data requirements. The study is now designated Acceptable. ██████████, 11/15/13.

### **Dog 28-Day Dietary Toxicity**

51950-0008 119471; “28-Day Range-Finding Toxicity (Feeding) Study with NTN 33893 Tech. in the Dog” (Author: Block, I., Research and Consulting Co., Itingen, Switzerland, Lab Project ID 99656, 10/9/87); NTN 33893 Tech. (Batch No. PT 2/86, 92.8% purity); 0, 200, 1000 and 5000 ppm in feed (pellet-form) to 2 dogs/sex/dose for 28 days; all dogs in 5000 ppm group died or killed for humane reasons before termination of study; treatment related signs for 5000 ppm group included ataxia, tremors and vomiting; no deaths or clinical signs at 1000 or 200 ppm; marked reductions in food consumption and body weight for dogs in 5000 ppm group that survived past day two; transient reductions in food consumption for dogs in 1000 ppm group; slight hepatocellular hypertrophy in one male and minimal thyroid follicular atrophy in one female both at 1000 ppm; no other abnormal effects reported; NOEL (M/F) = 200 ppm (7.3 mg/kg/day), based on minimal morphological alterations; Supplemental (Patterson, 4/7/93)

### **Dog Subchronic Oral Toxicity**

\*\*51950-0006 119468; “NTN 33893 Technical Subchronic Toxicity Study on Dogs in Oral Administration (Thirteen-Week Feeding Study)” (Author: Ruf, J., Bayer AG, Wuppertal, West Germany, Lab. Project ID #100176, 2/2/90); 82-1; NTN 33893 Tech. (Batch No. 180587, 95.3% purity); 0, 200, 600, 1800 ppm (reduced to 1200 ppm on week 4 due to low food consumption) in feed (mash-form) to 4 dogs/sex/dose for 13 weeks; all dogs survived the treatment period; palatability of the diet containing the two highest dose was an issue; in the high dose group there was substantial reduction in food consumption and decrease body weight gain until the dose was lowered when the rate of body weight gain was similar to all other groups; trembling and tremors were reported for the 600 and 1800/1200 ppm groups for the first 5 weeks of the study; no other abnormalities were reported; NOEL (M/F) = 200 ppm (based on trembling and tremors, see Worksheet Discussion); acceptable, with no adverse effects. (██████████, 4/8/93).

### **Rabbit 21-Day Dermal Toxicity Study**

\*\*51950-0007 119470; “Study for Subacute Dermal Toxicity in the Rabbit” (Author: W. Flucke; Bayer AG, Department of Toxicology, Wuppertal 1, West Germany; Report No. 100688; 6/11/90); NTN 33893 (Batch No. 180587; 95.0% imidacloprid), moistened with 2% Cremophor EL solution before application; 0 (vehicle), 1000 mg/kg/day; 5 HC:NZW rabbits/sex/dose group; animals were exposed under a porous patch for 6 hrs/d, 5 d/wk for 3 wks; no mortality or clinical signs of toxicity; no skin irritation or toxicologically significant findings; no adverse effects; NOEL ≥ 1000 mg/kg/day; Acceptable. (██████████ and ██████████, 4/19/93)

## CHRONIC STUDIES

### Combined Chronic and Carcinogenicity, Rat

\*\* 009,-010,-011; 119472, 119473, 119475; Chronic Toxicity and Cancerogenicity Studies on Wistar Rats (Administration in Food over 24 Months), (Authors: R. Eiben, G. Kaliner; 831; Rat; Bayer AG, Dept. of Toxicology, D-56 Wuppertal 1, West Germany; Report Nos. 100652, 101931, 102658; 9/6/91; NTN 33893 Technical (94.3% purity); 60 animals/sex/group; Doses: (Study #1)-0, 100, 300, 900 ppm, (Study #2)-0, 1800 ppm; Mortality: (104 wks)-O (M:16/100, F:26/100), 100 (M/F:6/50), 300 (M:6/50, F:10/50), 900 (M:6/50, F:13/50), 1800 (M:5/50, F:10/50); Clinical Observations: no treatment-related signs; weight gain reduced in 1800 ppm group (M: 5%), (F: 11%); Hematology: no treatment effect; Serum Chemistry: increased alkaline phosphatase activity (F, 1800 ppm) at 6, 12, 18 months; Gross Pathology: no treatment-related lesions; Histopathology: (non-neoplastic lesions) increased incidence of mineralized particles in colloid of thyroid gland, (neoplastic) cholangiocellular carcinoma in livers of 2 males (1800 ppm); possible adverse effect: cholangiocellular carcinoma; NOEL: 100 ppm, based on the incidence of mineralized particles in the thyroid glands of males in the 300 ppm group; Study acceptable. (██████████), 4/7/93)

51950-0116 122745 Bomhard et al., 1986, spontaneous tumor incidences from 11 lifetime rat studies. There were 962 males and 968 females evaluated. Of these, one male and 3 females had cholangioma/cystadenoma (p. 41). This background gives some support for Dr. Moore's designation of the above combined rat study as warranting a "possible adverse effect" designation based on incidence of cholangiocellular carcinoma in males, despite lack of statistical significance in that study. ██████████, August 15, 2012

### Chronic, Dog

\*\* 013; 119478; "52-Week Oral Toxicity (Feeding) Study with NTN 33893 Technical in the Dog," Allen, T. R., *et al.*, Research and Consulting Co., Itingen, Switzerland, Lab Project ID 100015, 10/19/89. NTN 33893 Tech. (Batch No. 180587, 94.9% purity); 0, 200, 500 and 1250/2500 ppm in feed (pellet-form) to 4 dogs/sex/dose for 52 weeks; the 1250 ppm dose was increased to 2500 ppm at week 17 due to the lack of apparent toxicity; no animals died during the study; there were no treatment-related effects on clinical signs, body weights, ophthalmoscopy, hearing, hematology or urinalysis; there was a slight but non-significant increase of liver weights in both sexes of the high dose group; there was also a slight increase in plasma cholesterol in females and an increase in liver cytochrome P450 in both sexes at the high dose; NOEL (M/F) = 500 ppm (based on increased liver cytochrome P450); Acceptable (██████████), 4/9/93).

### Oncogenicity, Mouse

\*\* 014,-015; 119479; 119480; Carcinogenicity Study on B6C3F1 Mice (Administration in the Food for 24 Months), (Author: B. Watta-Gebert); 832; Mouse; Bayer AG, Department of Toxicology, D-56 Wuppertal 1, West Germany; Study Nos. 100693, 101929; 1/28/91, 10/24/91; NTN 33893 Technical (purity: 95.3%); 60 animals/sex/group; Doses: (Study #1) 0, 100, 330,1000 ppm, (Study #2) 0, 2000 ppm; Mortality: 0 (M:9/100, F:26/100), 100 (M:6/50, F:7/50), 330 (M:3/50, F:9/50), 1000 (M:8/50, F:9/50), 2000 (M:17/50, F:14/50); Clinical Observations: no treatment-related effects; weight gain reduced (2000 ppm) (M: 29%, F: 26%); Hematology: reduced WBC count (2000 ppm) (1000 ppm, F only); Serum Chemistry: alk. phosphatase activity increased (2000 ppm), cholesterol level decreased (2000 ppm), urea level decreased (2000 ppm, M only); Gross Pathology: no treatment-related lesions; absolute liver, brain, lung, spleen, kidney, and adrenal gland (F only) weights decreased (2000 ppm), liver (F only, 1000 ppm); relative liver, spleen weights decreased (F only, 2000 ppm); Histopathology: slight periacinar hepatocytic

hypertrophy (M only, 2000 ppm); mineralization in the thalamus (F only, 2000 ppm); no treatment-related incidence of neoplasms; no adverse effect identified; NOEL: 1000 ppm, (estimated compound intake: 143.1 mg/kg/day), based on reduced weight gain and increased mortality of animals in 2000 ppm group; Study acceptable. (██████, 4/9/93)

016; 119481; Pilot Range-Finding Study for a Cancerogenesis Study on B6C3F1 Mice, (Author: R. Eiben); 821; Mouse; Bayer AG, Department of Toxicology, D-56 Wuppertal 1, West Germany; Report No. 99808; 10/24/88; NTN 33983 Technical (purity: 92.8%); 10 animals/sex/group; Doses: 0, 120, 600, 3000 ppm; Mortality: 0 (M/F:0/10), 120 (M:1/10, F:0/10), 600 (M:1/10, F:0/10), 3000 (M/F:7/10); Clinical Observations: poor appearance (3000 ppm), significant reduction of body weight gain (3000 ppm); Hematology: no treatment-related effects; Serum Chemistry: elevation of alkaline phosphatase (3000 ppm); Gross pathology: no treatment-related lesions, reduced absolute brain, heart, liver, kidneys, spleen (F only), and adrenals (F only) weights (3000 ppm), reduced relative liver (F only), heart, and spleen (F only) weights (3000 ppm); Histopathology: no treatment-related lesions; target organ not identified; no possible adverse effect identified; NOEL: 600 ppm, (estimated daily intake: 85.7 mg/kg/day), based on poor appearance, reduced weight gain and increased mortality in the 3000 ppm group; Study supplemental. (██████, 4/8/93)

## GENOTOXICITY

### Gene Mutation

\*\*51950-020; 119497; mutagenicity; 842; “NTN 33893; Reverse Mutation Assay (*Salmonella typhimurium* and *Escherichia coli*)”; author, M. Watanabe; Nihon Tokushu Noyaku Seizo K. K., Basic Research Division, Hino Institute, Toxicological Research Laboratory, Japan; 1/17/91; report #101276; Imidacloprid Technical (NTN 33893; 93.7% purity); doses (+/- S9 microsomes): 312.5, 625, 1250, 2500, & 5000 µg/plate, triplicate cultures, 2 independent trials; *S. typhimurium* tester strains TA98, 100, 1535, & 1537 and *E. coli* strain WP2/uvrA; positive controls +/- S9 were successful in all instances; 48 hr exposure; no adverse effects: there was no evidence for mutagenicity (*i.e.* an increase in revertants arising in low-histidine or low tryptophan medium) in any tester strain, regardless of the presence or absence S9 activating microsomes; Acceptable. (██████, 4/8/93)

\*\* 51950-020; 119499; mutagenicity; 842; “NTN 33893; Salmonella/Microsome Test to Evaluate for Point Mutagenic Effects”; Bayer AG, Institute of Toxicology, FRG; author, B.A. Herbold; 1/6/89; report #98570; Imidacloprid Technical (NTN 33893; 95.0% purity); *S. typhimurium* strains TA 98, TA 100, TA 1535, & TA 1537; doses, Test #1 (+/- 30% S9): 0, 20, 100, 500, 2500, & 12,500 µg/plate; Test #2 (-S9, +10% S9, & +30% S9): 0, 775, 1550, 3100, 6200, & 12,400 µg/plate; slight cytotoxicity at high dose based on titer determinations in high-histidine agar; no adverse effects: no evidence for mutagenicity (*i.e.* an increase in revertants arising in low-histidine agar) in any tester strain, regardless of the presence or absence S9 microsomes and despite the success of the positive control compounds; Acceptable. (██████, 4/14/93)

## 51950-0210 143405 “Mutagenicity evaluation using the Salmonella/microsome test,” Bayer AG Fachbereich Toxikologie Wuppertal, Germany, 08/01/1992. This volume is currently not available from the DPR Library. A report from the USDA (2005, page 44 of website [http://www.fs.fed.us/foresthealth/pesticide/pdfs/122805\\_Imidacloprid.pdf](http://www.fs.fed.us/foresthealth/pesticide/pdfs/122805_Imidacloprid.pdf)) indicates that “The available data indicate that neither imidacloprid nor its nitrosoimine metabolite, WAK 3839, are mutagenic or

genotoxic.” Thus the study has presumably been evaluated by federal government reviewers, and has been found negative. [REDACTED] no worksheet at this time, August 14, 2012.

\*\*51950-020; 119498; mutagenicity; 842; “NTN 33893; Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay” author, H. Lehn; Bayer AG, Institute of Toxicology, FRG; 1/6/89; report #98584; Imidacloprid Technical (NTN 33893; 95.2% purity); doses ranged between 0-125 µg/ml in the absence of S9 activating microsomes and between 0-1222 µg/ml in the presence of S9; 5 hr exposure; cytotoxicity was evident directly after treatment with 70 and 80 µg/ml test article, +/- S9, respectively; no adverse effects: no increase in 6-thioguanine resistance was measured under any condition, thus NTN 33893 is not considered mutagenic in this system; Acceptable. ([REDACTED], 4/8/93)

### **Chromosomal Aberration**

\*\* 51950-020; 119500; structural chromosome aberration; 843; Chinese hamsters; “NTN 33893; In Vivo Cytogenetic Study of the Bone Marrow in Chinese Hamster to Evaluate for Induced Clastogenic Effects”; Bayer AG, Institute of Toxicology, FRG; author, B. A. Herbold; 11/24/89; report #100021; Imidacloprid Technical (NTN 33893; 94.6% purity); dose: 2000 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 30 mg/kg cyclophosphamide; animals sacrificed at 6, 24, & 48 hr post dose (positive & negative controls were sacrificed at 24 hr only); 5/sex/sacrifice group; deaths: 4/34 animals treated with test article from acute toxicity; no variations of biological significance were seen in chromosomal integrity among all treatment groups and negative controls; positive controls exhibited large increases in % metaphases with aberrations; no adverse effects: NTN 33893 is not clastogenic in this assay under the conditions tested; Acceptable. ([REDACTED], 4/15/93)

\*\* 51950-020; 119501; structural chromosome aberration; 843; “NTN 33893; In Vitro Cytogenetic Study with Human Lymphocytes for the Detection of Induced Clastogenic Effects”; Bayer AG, Institute of Toxicology, FRG; author, B. A. Herbold; 6/16/89; report #99262; Imidacloprid Technical (NTN 33893; 95.2% and 99.8% purity, 1st & 2nd experiments, respectively); cells freshly isolated from 1 male & 1 female volunteer; doses (-/+ S9 microsomes), Expt. #1: 0, 50, 500, & 5000 µg/ml; Expt. #2: 0, 1300, 2600, & 5200 µg/ml; cytotoxicity, indicated by a decline in mitotic index, was most prominent w/o S9 (declines to 64% of control @ 500 µg/ml in Expt. #1 and 41.4% of control @ 1300 µg/ml in Expt. #2) and was only weakly apparent w/S9; clastogenesis, indicated mainly by the appearance of chromosomal gaps & breaks, was also most prominent w/o S9 (metaphases w/aberrations excluding gaps increased from 3.0% in controls to 14% @ 5000 µg/ml w/no effect @ 50 & 500 µg/ml in Expt. #1 and from 2.0% to 10.0 and 28.0% @ 1300 & 2600 µg/ml in Expt. #2); only weak clastogenic effects seen in the presence of S9; possible adverse effects: NTN 33893 is clastogenic in this assay under the conditions tested; Acceptable. ([REDACTED], 4/16/93)

\*\* 51950-020; 119503; structural chromosome aberration; 843; Mouse; “NTN 33893; Micronucleus Test on the Mouse to Evaluate for Clastogenic Effects”; author, B. A. Herbold; Bayer AG, Institute of Toxicology, FRG; 6/27/88; report #102652; Imidacloprid Technical (NTN 33893; 95.3% purity); dose: 80 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide; animals sacrificed 24, 48, & 72 hr post dose; 5/sex/sacrifice group; no adverse effects: no test article-induced statistically significant increase over negative controls was observed in the number of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; no statistically significant alterations occurred in the ratio of polychromatic to normochromatic cells; Acceptable. ([REDACTED], 4/19/93)

\*\* 51950-020; 119504; structural chromosome aberration; 843; Mouse; "Mouse Germ-Cell Cytogenetic Assay with NTN 33893"; author, W. Volkner; Cytotest Cell Research GmbH & Co. KG, In den Leppsteinswiesen 19, Robdorf, FRG; 5/22/90; report #102654; Imidacloprid Technical (NTN 33893; 94.1% purity); dose: 80 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 10 mg/kg doxorubicin sulfate HCl dosed in saline; animals sacrificed 6, 24, & 48 hr post dose; 6/males/sacrifice group (only 5 were evaluated); spermatogonia were isolated from both testes and prepared on slides; despite a successful positive control, the test article failed to induce any biologically relevant increase in spermatogonial chromosome aberrations; neither the positive control nor the test article had an effect on mitotic index; no adverse effects: under the conditions tested, NTN 33893 is neither clastogenic nor cytotoxic to mouse spermatogonia; Acceptable. (██████████ 4/20/93)

\*\* 51950-020; 119505; other genetic effects; 844; "Clastogenic Evaluation of NTN 33893 in an In Vitro Cytogenetic Assay Measuring Sister Chromatid Exchange in Chinese Hamster Ovary (CHO) Cells"; author, R. D. F. M. Taalman; Hazleton Biotechnologies, Landjuweel, Veenendaal, The Netherlands; 4/21/88; report #102655; Imidacloprid Technical (NTN 33893); 95.2% purity; doses, -S9, Trial I: 16.7, 50, 166.7, & 500 µg/ml; Trial II: 100, 250, 500, & 1000 µg/ml; +S9, Trial I: 166.7 & 500 µg/ml and 1.7 & 5.0 mg/ml; Trial III: 500 µg/ml and 1, 2, & 3 mg/ml; Trial II/-S9 and Trial III/+S9 gave results indicating a dose-dependent rise in SCE/diploid cell (4, 44, 56, & 96% over solvent control for Trial II/-S9 and 0, 8, 28, & 70% over solvent control for Trial III/+S9); cytotoxicity was present at concentrations above (and including) 500 µg/ml -S9 and at 3 mg/ml +S9; possible adverse effects: NTN 33893 induces SCE in CHO cells in the absence and presence of S9 under the conditions tested; Acceptable. (██████████ 4/21/93)

"An *In Vivo* Assay of the Mutagenic Potential of Imidacloprid Using Sperm Head Abnormality Test and Dominant Lethal Test"; (P. Bagri, V. Kumar, A.K. Sikka P. Bagri, V. Kumar, A.K. Sikka; Department of Veterinary Pharmacology and Toxicology and Department of Animal Genetics and Breeding, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India; Drug and Chemical Toxicology 38: 342-348 (2015)); The researchers employed two *in vivo* assays to assess the potential of imidacloprid to induce chromosomal aberrations in germ cells of male mice, the sperm head abnormality test (SHA) and the dominant lethal test (DLT). In the SHA test, 18 male Swiss mice/group were dosed orally by gavage with 0 (vehicle: aqueous 3% gum acacia), 5.5, 11 or 22 mg/kg/day of Imidacloprid Technical (purity:>98%). Six animals/group/time point were euthanized after 7, 14 and 28 days of treatment. The caudae epididymides were dissected and the sperm cells were isolated. The cells were stained with 1% eosin-Y and mounted on a slide. Two hundred sperm/animal were assessed for abnormal heads. In the DLT 18 males/group were dosed orally by gavage with 0, 5.5, 11 or 22 mg/kg/day of imidacloprid for 28 days. At 1, 3 and 6 weeks post-final dose, 6 males/group/time point were each housed with an untreated female for one week. The females were euthanized 17 days after the initiation of the mating period. Their uteri were dissected and each implant was classified as to whether it as a normally healthy fetus or a mole (a mole was identified as a defective embryo that either was not implanted or died soon after implantation). In the SHA test, there was a dose-related increase in the frequency of abnormal sperm heads after 14 and 28 days of treatment. These results were deemed to be statistically significant. However the inclusion of a positive control in the study would have provided confirmation that the assay itself was sufficient to identify the effect under consideration. In the DLT, the assay results did not indicate a reduction in the number of viable fetuses over the treatment range for the 3 time points which were assayed. There was a minimal increase in the number of moles noted for the 22

mg/kg treatment group after mating at 6 weeks post-final dose. Was this increase due to the increased percentage of abnormal sperm heads noted for that treatment group after 28 days of treatment? Overall the study results do not provide sufficient data to establish a treatment-related effect indicative of chromosomal aberration. Summary report. (██████, 4/6/23)

#### DNA Damage or Miscellaneous Effects

\*\*51950-020/158; 119502/128284; other genetic effects; 844; Chinese hamsters; “NTN 33893; Sister Chromatid Exchange in Bone Marrow of Chinese Hamsters In Vivo”; author, B. A. Herbold; 6/16/89 (original), 11/11/93 (supplement); Report #99257-1; Imidacloprid Technical (NTN 33893; 95.0% purity); doses: 0, 500, 1000, & 2000 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg b.w.); positive control: 10 mg/kg cyclophosphamide (CP); animals sacrificed 24 hr post dose, 2 hr after colcemid treatment to arrest cells in metaphase; 5/sex/dose (50 metaphases/animal analyzed for SCE); marrow preparations made from the femur; no deaths; no toxic clinical signs; cytotoxicity was present at 1000 and 2000 mg/kg (mitotic index declined at both doses to 83.3% of controls); no change in proportion of cells in 1st, 2nd, & 3rd metaphases indicating no effect on cell cycling; sister chromatid exchange rate was also unaffected (SCE mean rate per metaphase was 2.01, 2.17, 2.28, & 2.41 for 0, 500, 1000, & 2000 mg/kg, respectively) despite successful positive control (SCE rate was 15.27 for 10 mg/kg CP,  $p < .01$ ); Acceptable. (██████, 4/19/93; revised from unacceptable with submission of individual animal data by ██████, 3/8/94)

\*\* 51950-020; 119506; other genetic effects; 844; “Sister Chromatid Exchange Assay in Chinese Hamster Ovary Cells”; author, D. L. Putnam & M. J. Morris; Microbiological Associates, Inc., Rockville, MD; 9/12/89; report #99676; Imidacloprid Technical (NTN 33893); 95.2% purity; doses, -S9: 25, 50, 100, 200, & 400  $\mu\text{g/ml}$ ; +S9: 157, 313, 625, & 1250  $\mu\text{g/ml}$ ; no adverse effects: no evidence for induction of SCE in the presence or absence S9 in this system despite cytotoxicity present at each dose tested; Acceptable. (██████ 4/21/93)

\*\* 51950-020; 119508; other genetic effects; 844; “Mutagenicity test on NTN 33893 in the Rat Primary Hepatocyte Unscheduled DNA Synthesis [UDS] Assay”; (author, M. A. Cifone; Hazleton Laboratories America, Inc., Kensington, MD, report #98573, 12/21/88); Imidacloprid Technical (NTN 33893); 95.2% purity; 5 trials, cells isolated from each of 2 rats/trial; Trials 1, 2, & 4 were non-functional; doses, Trial 3: 5 (Rat #2 only), 10, 25, 50, 100, 250, & 500  $\mu\text{g/ml}$ ; doses, Trial 5: 50, 100, 250, 375, 500, 750  $\mu\text{g/ml}$ ; higher concentrations not analyzed because of excessive toxicity; UDS assessed by autoradiographic determination of  $^3\text{H}$ -thymidine incorporation; criteria for positive UDS response (net nuclear grain count more than 6 above negative controls, % nuclei  $w \geq 6$  grains was at least 10% of the population more than controls, % nuclei  $w \geq 20$  grains exceeds 2% of the population) not fulfilled at any dose (the positive control, 2-acetyl aminofluorene, was successful); however; there was evidence for a weakly positive response at high doses; no adverse effects; Acceptable. (██████ 4/23/93)

\*\* 51950-020; 119507; other genetic effects; 844; *Bacillus subtilis*; “NTN 33893; Rec-assay with Spores in the Bacterial System”; author, M. Watanabe; Nihon Tokushu Noyaku Seizo K. K., Basic Research Division, Hino Institute, Toxicological Research Laboratory, Japan; 6/18/90; report #101275; Imidacloprid Technical (NTN 33893; 94.7% purity; doses (-/+ S9): 312.5, 625, 1250, 2500, 5000  $\mu\text{g/disk}$ ; positive controls, mitomycin C (-S9) and 2-aminoanthracene (+S9) successfully generated large differences in growth inhibition zone between the Rec+ and Rec- *B. subtilis* strains H17 and M45, respectively, indicating a positive gene damaging effect; no adverse effects: no test article-induced

differences were observed in growth inhibition zones between the 2 strains, thus no damage occurred which a Rec+ DNA repair system might have remedied; Acceptable. (██████████ 4/22/93)

\*\* 51950-020; 119509; other genetic effects; 844; "NTN 33893; Test on *S. cerevisiae* D7 to Evaluate for Induction of Mitotic Recombination"; author, B. A. Herbold; Bayer AG, Institute of Toxicology, FRG; 6/27/88; report #102653; Imidacloprid Technical (NTN 33893); 95.3% purity; 2 trials; single test tube/dose replated onto 10 plates in complete agar medium to detect mitotic crossing over by colony color or in tryptophan-deficient agar to detect mitotic gene conversion; doses: 0, 625, 1250, 2500, 5000, 10000 µg/ml; positive controls: -S9, methyl methane sulfonate; +S9, cyclophosphamide; no adverse effects: since there were no changes in the numbers of red or pink colonies or in the ability to grow in tryptophan-deficient medium as compared to negative controls, there was no evidence of the occurrence of recombination events, either in the form of crossing over or gene conversion; positive controls stimulated both types of recombination; Acceptable. (██████████ 4/26/93)

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## REPRODUCTIVE TOXICITY

### Rat

\*\* 019; 119496; Multiple Generation Reproduction Study in Rats, (Authors: P. Suter *et al.*); RCC, Research and Consulting Company AG, Itingen, Switzerland; Study No. 100647; 6/21/90; NTN 33893 Technical (purity: 95.3%); P generation: 30 animals/sex/group, F1B generation: 26 animals/sex/group; 2 litters/generation; Dose: 0, 100, 250, 700 ppm; Mortality: (P) 0 (M:0/30, F:2/30), 100 (M:0/30, F:1/30), 250 (M/F:0/30), 700 (M/F:0/30), (F1B) 0 (M/F:0/30), 100 (M/F:0/30), 250 (M:1/30, F:0/30), 700 (M/F:0/30); Clinical observations: decreased body weight gain (F0-700 ppm M, F1B F); Hematology: no treatment-related effects; Clinical Biochemistry: increased O-demethylase activity (F1B-250 ppm F, 700 ppm M, F), N-demethylase activity (F1B-700 ppm M), and cyt. P450 activity (F1B-700 ppm, M); Necropsy: no treatment-related lesions, no effect on organ weights; Histopathology: no treatment-related lesions; Reproductive factors: no treatment-related effects on fertility index, litter size; Developmental factors: no treatment-related abnormalities, decreased weight gain (F1A, F1B, F2A, F2B-M, F, 700 ppm), no treatment-related effect on gestation index, viability index, or lactation index; no adverse effects identified; NOEL: (parental) 700 ppm, (reproductive) 700 ppm, (developmental) 250 ppm (based on decreased weight gain for pups, 700 ppm); Study acceptable. (██████████, 4/14/93)

51950-0116 122744 The range-finding study to determine the appropriate dosing levels for 51950-0019 119496, above. The parental generation received the test material in the diet at dose levels of 0, 20, 100, and 500 ppm. Treatment-related effects of reduced female (P) body weight gain in the pre-pairing period and reduced pup weights were noted for the 500 ppm dose group. This pilot study was noted in ██████████ review of the primary study.

## DEVELOPMENTAL TOXICITY

### Rat

\*\* 017; 119482; Embryotoxicity Study (including Teratogenicity) with NTN 33893 Technical in the Rat (Authors: H. Becker, *et al.*); 833; Rat; RCC, Research & Consulting Company AG, Itingen, Switzerland; Study No. 98571; 1/8/92; NTN 33893 Technical (purity: 94.2%); 25 females/group; Doses 0, 8.9, 25.9, 94.1 mg/kg/day (analytical), test material administered by gavage from day 6 post coitum through day 15; No mortality; Clinical observations: no treatment-related signs, mean food consumption and body weight



gain decreased during treatment period (94.1 mg/kg/day); Necropsy: no treatment-related lesions; Developmental: high percentage of male fetuses, increased incidence of wavy ribs (94.1 mg/kg/day); Maternal NOEL = 25.9 mg/kg/day (based on decreased body weight gain and reduced food consumption of the 94.1 mg/kg/day treatment group; Developmental NOEL = 25.9 mg/kg/day (based on increased incidence of wavy ribs in the fetuses of the 94.1 mg/kg/day treatment group); Study acceptable. (██████████, 4/19/93)

51950-114 112742 (RCC Project 083507). This is the pilot study to 51950-117 119482, above. Dams were treated with 0, 50, 100, or 150 mg/kg of the test article by gavage starting on day 6 and going through day 15 post coitum. The dams exhibited as dose-related reduction in food consumption from day 6 to 10 p.c. which was reflected in a body weight loss or no weight gain for the two higher dose groups. There were no treatment-related effects upon development of the offspring. Examined by ██████████ in context of the primary study review: no DPR worksheet.

### **Rabbit**

\*\* 018; 119484; Embryotoxicity Study (including Teratogenicity) with NTN 33893 Technical in the Rabbit, (Authors: H. Becker, K. Biederman); 833; Rabbit; RCC, Research and Consulting Company AG, CH 4452 Itingen, Switzerland; Study No. 98572; 1/8/92; 16 females/group; Doses: 0, 7.0, 20.5, 64.3 mg/kg/day (analytical), doses administered by gavage from day 6 post coitum through day 18; Mortality: 0 (0/16), 7.0 (0/16), 20.5 (0/16), 64.3 (2/16); Clinical observations: reduced food consumption, body weight loss day 6 to 19, one abortion (64.3 mg/kg/day), reduced body weight gain day 6 to 19 (20.5 mg/kg/day); Necropsy: no treatment-related lesions; Developmental: one abortion, two total resorptions, increased post-implantation loss, reduced mean fetal weight (64.3 mg/kg/day); no adverse effects; Maternal NOEL = 20.5 mg/kg/day (based on mortality of dams, decreased body weight gain for 64.3 mg/kg/day treatment group); Developmental NOEL = 20.5 mg/kg/day (based on increased post-implantation loss, decreased fetal weight of the offspring in the 64.3 mg/kg/day treatment group); Study acceptable. ██████████, 4/16/93).

51950-115 122743 (RCC Project 083520), Nov. 1, 1988. This was a range-finding study to establish the dosing levels for Record No. 119484, above. The dams were dosed with 0, 50, 100, or 150 mg/kg/day of the test material by gavage from day 6 post coitum through day 18 p.c. All of the dams treated with the highest dose died by day 11 p.c. In the remaining two dose groups, food consumption was reduced during the dosing period with a corresponding loss in weight. In the 100 mg/kg group, a treatment-related increase in embryonic resorptions was reported. (Taken from above review of primary study by ██████████, 4/16/93).

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## **NEUROTOXICITY**

### **Acute Neurotoxicity, Rat**

51950-0472, -0473; 209391, 209392; "An Acute Oral Neurotoxicity Screening Study with Technical Grade Imidacloprid (NTN 33983) in Rats"; (L.P. Sheets; Miles Inc., Agriculture Division, Toxicology, Stilwell, KS; Study Nos. 106348, 106348-1; 2/16/94 and 6/7/94); Two acute neurotoxicity studies were performed. In the 1<sup>st</sup> study, eighteen Sprague-Dawley rats/sex/group were dosed orally by gavage with 0, 42, 151 or 307 mg/kg of Imidacloprid Technical (NTN 33893 technical, batch no. 2030030, purity: 98.8% (8/92)). Six animals/sex/group were identified as the satellite animals and used for clinical pathology

testing. In the 2<sup>nd</sup> study, 12 females/group were likewise dosed orally with 0 or 20 mg/kg of the test material (same batch no., purity: 98.6% (4/94)). In the 1<sup>st</sup> study, 4 males and 10 females in the 307 mg/kg group died within two days of dosing. In the functional observational battery (FOB) performed 90 minutes after dosing, some of the 307 mg/kg group animals displayed tremors and incoordination in their gait in the home cage and open field tests. In the home cage, some of these animals exhibited greater or less than normal activity levels. In the open field test, the animals were generally more sluggish in their movements. The mean frequency of rearing was also reduced for both sexes of this group (M: NS, F: <0.05). In the reflex/physiologic testing, some of the animals in the high dose had no reaction to touch, auditory or pinch stimuli. For the 151 mg/kg group females, one of the 12 animals exhibited tremors in the FOB on Day 0. Mean hindlimb strength was lower for the 307 mg/kg males on Day 0. Mean motor and locomotor activities for both sexes in the 151 and 307 mg/kg groups were lower than those of the control on Day 0. Although some of the values for the hematological and clinical chemical parameters in the 307 mg/kg group were significantly different from those of the control, these differences were not considered to be toxicologically relevant. In the necropsy examination, the mean absolute brain weight for the 307 mg/kg males was less than that of the control ( $p < 0.05$ ), the relative weights were not significantly different. No treatment-related effects were noted in the 2<sup>nd</sup> study. Possible adverse effect: tremors and other signs of neurotoxicity; NOEL (M/F): 42 mg/kg (based upon the decreased motor and locomotor activity levels and presence of tremors in the 151 mg/kg treatment group); Study acceptable. (██████████, 3/3/04)

### **90-Day Neurotoxicity, Rat**

\*\*51950-0471; 209390; "A Subchronic Dietary Neurotoxicity Screening Study with Technical Grade Imidacloprid (NTN 33983) in Fischer 344 Rats"; (L.P. Sheets; Miles Inc., Agriculture Division, Toxicology, Stilwell, KS; Study No. 106356; 6/13/94); Eighteen Fischer 344 rats/sex/group received 0, 140, 963 or 3027 ppm of Imidacloprid Technical (NTN 33893 technical, batch no. 2030030, purity: 97.6% (3/93)) in the diet for 13 weeks ((M) 0, 9.3, 63.3, 196 mg/kg/day, (F) 0, 10.5, 69.3, 213 mg/kg/day). Six animals/sex/group were used as satellite animals of use in the hematology and clinical chemistry evaluations. No deaths occurred during the study. The mean body weights and food consumption of both sexes in the 963 and 3027 ppm groups were lower than those of the control group ( $p < 0.05$ ). In the Functional Observational Battery (FOB), although the mean hindlimb grip strength of the 3027 ppm males was lower after 8 weeks of treatment ( $p < 0.05$ ) and a greater number of these males had a slightly uncoordinated righting reflex at 13 weeks ( $p < 0.05$ ), these results did not indicate a consistent effect and were considered to be incidental. Otherwise, no other effects were evident in the FOB. In the clinical chemistry evaluation, serum triglyceride concentrations were lower for both sexes in the 3027 ppm group at both 4 and 13 weeks ( $p < 0.05$ ). Mean phosphate levels were reduced for both sexes in the 3027 ppm group at 4 weeks and for the males in that group at 13 weeks ( $p < 0.05$ ). The mean albumin concentrations for the 3027 ppm females were lower than those of the controls at both 4 and 13 weeks ( $p < 0.05$ ). Although the mean values of other parameters for the 3027 ppm group demonstrated an increase or decrease over the values for the controls, the observed effects were not consistent over the course of the study or were doubtful toxicological significance. There were no treatment-related effects evident in the hematology results, the necropsy or the histopathology examinations. No signs of neurotoxicity were noted. No adverse effect indicated. Subchronic NOEL (M/F): 140 ppm ((M) 9.3 mg/kg/day, (F) 10.6 mg/kg/day) (based upon lower mean body and food consumption of the 963 ppm group). Study acceptable. (██████████, 3/5/04)

### Developmental Neurotoxicity, Rat

51950-0474 209393 Sheets, L. P., "A developmental neurotoxicity screening study with technical grade Imidacloprid in Wistar Rats," Bayer Corp., Stilwell, KS, 9/14/01. Study # 99-D72-DV: Bayer Report No. 110245. Thirty CrI:W(HAN)BR mated females/group were dosed in diet with 0, 100, 250, or 750 ppm imidacloprid (98.2% purity) throughout gestation and lactation (ending lactation day 21). Estimated mean gestation exposures were 8.2, 19, and 57 mg/kg/day. Estimated mean exposures during lactation days 0-14 were 0, 15, 36, and 104 mg/kg/day. At least 21 litters per group were of sufficient size to maintain offspring until sacrifice at about postnatal day (PND) 75. Maternal NOEL = 250 ppm, based on transient reduction in food consumption during lactation days 0-7. Developmental toxicity NOEL cannot be determined because intermediate groups were not evaluated in the presence of a conspicuous change in 750 ppm in morphometric measurements (see below). Most endpoints other than the morphometric measurements were evaluated in intermediate dose levels, and none of these found treatment effects at 250 ppm. Findings at 750 ppm in offspring were reduced mean pup weight (5 g) at PND 21 weaning, reduced motor activity in PND 17 males and females and in PND 21 females, modest reductions in motor and locomotor activities during the first recording interval in PND 60 males (suggesting a slight reduction in exploratory activity in a novel environment), and a substantial reduction in the thickness of the corpus callosum in PND 11 females only (not reflected in PND 75 rats of either sex). Study is not acceptable, and appears not to be upgradeable. The apparent corpus callosum change in 750 ppm females at PND 11 indicates a need to analyze intermediate groups. The statistic procedures for PND 11 morphometric measurements need to be examined. Morphometric measurements should be performed in intermediate groups wherever an effect is statistically significant at 750 ppm. Cited positive control method validation studies contemporary with this study are requested. See discussion of DPR review for details on concerns about study conduct and report presentation. Other than these issues, this study addressed the full scope of evaluations that pertain to developmental neurotoxicity studies. [REDACTED] 3/24/04.

\*\* 51950-0998; 344610; "Developmental Neurotoxicity Study of Imidacloprid Technical in Wistar Rats"; (M.V. Patel; Jai Research Foundation, Department of Toxicology, Dist. Valsad, Gujarat, India; Study No. 8796; 1/12/10); Twenty seven-mated female Wistar rats/group received 0, 100, 250 or 750 ppm of Imidacloprid Technical (batch no. 2003/09; purity: 99.42%) in the diet from gestation day 0 through lactation day 21 (a.i. uptake: gestation, 0, 7.4 to 8.9, 17.6 to 22.2, 39.4 to 58.6 mg/kg/day, lactation: 0, 12.1 to 21.7, 27.7 to 54.1, 78.3 to 163.1 mg/kg/day). Offspring from 25, 24, 24, and 25 litters in the control and three treated groups, respectively, were assessed neurologically for up to 70 days post-natal in a functional observational battery (FOB), motor activity assessment, motor and sensory function, auditory startle response, and Morris Maze learning and memory assessments. The neuropathologic examination and morphometric analysis of the brain from the F1 generation were performed. No treatment-related maternal mortalities occurred during the treatment period. The mean body weights and food consumption of the 750 ppm dams were less than the control group values over the course of the treatment period. The maternal FOB assessment did not identify any treatment-related effects. There was no treatment-related effect on the live birth, viability or lactation indices. The mean body weight of the 750 ppm pups and young adult animals of both sexes was less than that of the control group throughout the study (the 750 ppm male pup weights were 12% less on post-natal day 4, increasing to 19% on day 21 and then diminishing to 11% on day 35 and 7% less by day 70; the 750 ppm female pup weights were 11% less than the control group on post-natal day 4, increasing to 17% on day 21, and then diminishing to 9% by day 35 and 6% on day 70). There was no treatment related effect on the time to physical development landmarks (unfolding of the pinna, hair growth, teeth eruption, eye opening, balano

preputial separation or vaginal opening). The pup FOB assessment did not reveal any apparent treatment-related effects on postnatal days 4, 11, 21, 35, 45 and 60. In the initial review, the behavioral ontogeny assessment (negative geotaxis, wire maneuver, and air righting reflex), which was measured on postnatal days 5 and 10, indicated a performance deficit by the 750 ppm pups of both sexes particularly on postnatal day 10. However, there was a concern in regard as to whether the noted effects may have been due to the lag in growth experienced by both sexes in the group during the treatment period. Upon further statistical analysis, it was shown that the lag in physical development of the 10-day old male pups did not significantly affect the performance of the individual animals in the negative geotaxis evaluation. Statistical significance was established for the male pups at the 250 and 750 ppm treatment levels ( $p < 0.05$  and  $0.01$ , respectively). Motor activity which was assessed on postnatal days 13, 17, 21, and 60 did not reveal any treatment-related effects. No treatment-related effects were noted in motor and sensory function evaluation and the basic habituation test which were performed on postnatal days 23 and 61. In the learning and memory test which was performed between postnatal days 24 and 28 and between 60 and 66 did not indicate any treatment-related deficit in learning or memory. In the brain morphometry, although some of the parameters indicated a significant difference between the 750 ppm group and the control group, there was no consistent difference in regard to the majority of the measurements in the postnatal day 11 cohort. In the 70-day cohort, the corpus callosum of both sexes in the 750 ppm group was larger than that of the control ( $p < 0.05$ ). The mean absolute and relative unfixed brain weights of the 11 and 70 day old pups were not affected by the treatment. The histological examination of the nervous tissues did not reveal any treatment-related lesions. Maternal NOEL: 250 ppm (17.6 to 22.2 mg/kg/day) (based upon the lower mean body weights of the 750 ppm dams); Rat Developmental Neurotoxic NOEL: 100 ppm (7.4 to 8.9 mg/kg/day) (based upon the treatment-related effect noted for the 250 ppm male pups in the negative geotaxis evaluation); Study acceptable. (██████████, 12/9/22, revised, ██████████, 1/23/24)

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## IMMUNOTOXICITY

\*\*51950-0835 274588; Kennel, P., "Imidacloprid: 28-day immunotoxicity study in the male Wistar rat by dietary administration," Bayer CropScience, Sophia Antipolis, France, Nov. 5, 2010. Laboratory Study # SA 09406. Ten male Wistar Rj:WI (IOPS HAN) rats/group were dosed in diet with 0, 150, 600, or 2400 ppm imidacloprid (technical, purity 98.5%). Estimated imidacloprid intake in treated rats was 12, 47, and 186 mg/kg/day. Positive controls received 3.5 mg/kg/day cyclophosphamide by gavage. NOEL = 600 ppm, based on 19% decrement in body weight, marked food consumption decrement at 2400 ppm (most marked at 54% of control consumption during week 1), and possible treatment response of piloerection and "wasted" appearance in one 2400 ppm rat. IgM was unaffected by imidacloprid treatment. Absolute reduction in thymic weight at 2400 ppm imidacloprid and relative increase in splenic weight at 2400 ppm imidacloprid appear to be incidental or associated with non-specific changes associated with body weight and food consumption effects at that dose level. Study is acceptable, and negative for immunotoxicity. ██████████, 11/18/13.

"Immunotoxic Effects of Imidacloprid Following 28 Days of Oral Exposure in BALB/c Mice"; (P.C. Badgular, S.K. Jain, A. Singh, J.S. Punia, R.P. Gupta, G.A. Chandratre; Department of Veterinary Pharmacology and Toxicology, Immunology Section, Department of Veterinary Microbiology, and Department of Veterinary Pathology, College of Veterinary Sciences, CCS Haryana Agricultural University, Hisar, India and Department of Veterinary Pathology, Bombay Veterinary College, Mumbai, India; Environmental Toxicology and Pharmacology 35: 408-418 (2013)); Six to 8 female Balb/c

mice/group were dosed orally by gavage with 0 (vehicle: aqueous 0.5% carboxymethyl cellulose), 2.5, 5.0 or 10 mg/kg/day of Imidacloprid technical (purity: >98%) for 28 days. Positive control groups were dosed with either 50 mg/kg of cyclophosphamide or 2 mg/kg of dexamethasone for 5 days. The humoral and cellular immune response was assessed. In the assessment of hemagglutinating antibody titer, each animal was injected intraperitoneally on day 21 of treatment with 0.3 ml of Sheep red blood cells (SRBC) ( $4.5 \times 10^8$  cells/animal). After 28 days of dosing with the test material, serum samples were drawn from each animal. When SRBCs were then added to the serum samples, the antibody titer to those cells was determined by serial dilution of the samples. In the delayed-type hypersensitivity (DTH) response assay, a second cohort of animals was treated in the same manner with the test material or 2 mg/kg of dexamethasone. On day 18 of treatment, each animal received a subcutaneous injection of 50  $\mu$ l of SRBC ( $10^8$  cells/animal). For the positive control group that was on day 2 of treatment. On day 28 the immune system of each animal was challenged by a 50  $\mu$ l injection of SRBC ( $10^8$  cells) into the foot pad. Swelling of the foot pad was assessed at 24 and 48 hours post-injection. Each foot pad was examined histologically in order to ascertain any cellular changes which may have occurred. In the lymphocyte proliferation assay, splenocytes were recovered from each study animal after 28 days of treatment, Triplicate cultures of splenocytes from each animal were incubated with or without phytohemagglutinin for 72 hours at 37° C. The number of proliferating cells was determined by treatment of the cells with tetrazolium salt XTT dye combined with phenazine methosulfate for an additional 3 to 4 hours. Absorbance was measured at 450 nm. In addition, at the conclusion of the 28-day treatment period, blood samples were collected from the orbital sinus. A differential white blood cell count was performed. The weights of the spleen, liver, kidneys and lungs were recorded and examined histologically. Splenic cellularity was measured. In the hemagglutinin antibody titer assay there was no treatment-related effect on the humoral immune response. For the delayed-type hypersensitivity, there was a treatment-related decrease in the foot pad thickness by 48 hours post-treatment for all of the treatment levels. Treatment also diminished the stimulation response to phytohemagglutinin. In the differential white blood cell count, there was no treatment-related effect upon the relative percentages of the different white blood cells. The relative spleen weights of the imidacloprid-treated groups were less than that of the control group (not statistically significant). Histological examination of the spleen revealed that there was a dose-related depletion of lymphocytes from the splenic white pulp. Examination of the foot pads indicated a strong local inflammatory response in the control group. This response was graded from strong to markedly muted in the 2.5, 5 and 10 mg/kg imidacloprid-treated groups, respectively. Overall treatment with imidacloprid resulted in no apparent effect on the humoral immune response. Treatment did affect the cellular immune system with diminution of the lymphocytic immune response (decreased delayed type hypersensitivity response and decreased stimulation index of T-lymphocytes to phytohemagglutinin). The 2.5 mg/kg treatment level did not produce a significant changes in the cellular and humoral immune responses. Summary report (██████████ 4/7/23)

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## ENDOCRINE DISRUPTOR STUDIES

Studies not required at this time.

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## SUPPLEMENTAL STUDIES

### Rat Acute Intraperitoneal Injection Toxicity

51950-0003 119464; “NTN 33893 Study For Acute Intraperitoneal Toxicity In Rats” (Author: Krötlinger, F., Bayer AG Department Of Toxicology, Wuppertal, West Germany; Report # 100689; 7/19/90); NTN 33893 (Purity = 94.2%) mixed with Cremophor EL and 0.9% NaCl solution; i.p. doses of (M) 10, 100, 160, 170, 180, 200, 250, 500 and (F) 10, 100, 150, 180, 200, 224 and 250 mg/kg; 5 rats/dose; mortalities (M) 0/5, 0/5, 0/5, 4/5, 4/5, 4/5, 5/5, 5/5 and (F) 0/5, 0/5, 1/5, 2/5, 2/5, 5/5 and 5/5 respectively; clinical observations included apathy, labored and accelerated breathing, decreased motility, periodic tremors and twitching; necropsy (dead rats) revealed lungs with isolated patches, livers occasionally pale and occasionally dark, spleens pale, small intestines reddened; LD50 (M) > 160 < 170 mg/kg and (F) = 186 (162-214) mg/kg; NOEL = 10 mg/kg; supplemental. (██████), 4/8/93)

## COMPANION ANIMAL STUDIES

51950-279; 151698; “Acute Oral Toxicity Evaluation of Imidacloprid (Advantage™) in Cats” (J. A. Shmidl and R. G. Arther, Bayer DeSoto Research Facility, Shawnee Mission, KS, Study # R-96-011, 11/11/96). Advantage (9.1% imidacloprid, Batch # 418004) was administered orally in gelatin capsules to 6 cats (2M/4F) at the label use rate (0.4 ml for cats weighing 9 lbs. or less and 1.0 ml for cats weighing > 9 lbs.). The control group of 6 cats (2 M/4F) received placebo capsules. Treated cats exhibited vomiting and depression. Similar clinical signs were also observed in placebo-treated cats. No mortalities were reported in either group at 14 days after dosing. No adverse effects indicated. Supplemental. (██████), 6/23/97).

51950-0258 145547 “Acute toxicity evaluation for dermal treatment of cats with Imidacloprid (BAY t 7391) Spot-On,” Miles Laboratories, Inc., 03/31/1995. Bayer Report No. 74579. Groups of 3 cats (mixed breeds, mixed gender per group) were administered this flea treatment formulation (10% imidacloprid) on the back-line (between shoulders) at 0 (placebo), or once with 50 mg/kg, or 3 daily applications at 50 mg/kg. Investigators evaluated body weight, clinical signs, and (at 13 days after first treatment) clinical chemistry and hematology. There were no other protocol parameters. The only noteworthy finding was a 6-fold increase in creatine phosphokinase in the 3-day treatment group only. Investigators considered this to be unrelated to the formulation, considering that other cat studies (with differing treatment regimens) have not repeated this finding. Supplementary data by design (not a study type normally reviewed by Medical Toxicology Branch). No adverse effects indicated. No worksheet. (██████), 8/14/12.

51950-0255 145544 “Acute toxicity evaluation for dermal treatment of dogs with Imidacloprid (Bay t 7391) Spot-On,” Miles Laboratories, Inc., 03/30/1995. Bayer Report No. 74580. Groups of 3 dogs (mixed breeds, 1 M and 2 F per group) were administered this flea treatment formulation (10% imidacloprid) on the back-line (between shoulders) at 0 (placebo), or once with 50 mg/kg, or 3 daily applications at 50 mg/kg. Investigators evaluated body weight, clinical signs, and (at 13 days after first treatment) clinical chemistry and hematology. There were no other protocol parameters. There were no observed treatment effects. Supplementary data by design (not a study type normally reviewed by Medical Toxicology Branch). No adverse effects indicated. No worksheet. (██████) 8/14/12.

51950-279; 151699; “Acute Oral Toxicity Evaluation of Imidacloprid (Advantage™) in Dogs” (J. A. Shmidl and R. G. Arther, Bayer DeSoto Research Facility, Shawnee Mission, KS, Study # R-96-011, 11/11/96). Advantage (9.1% imidacloprid, Batch # 418004) was administered orally in gelatin capsules to 6 Beagle dogs (3M/3F) at the labeled use rate (1.0 ml for dogs weighing 11 to 20 lbs.; 2.5 ml for 21 to 55 lbs. and 5.0 ml for dogs weighing > 55lbs). The control group of 6 Beagle dogs (3M/3F) received placebo capsules. Treated dogs exhibited vomiting at 1 hour following administration. However, placebo-treated dogs did exhibit any clinical signs. No mortalities were reported in both groups at 14 days after dosing. No adverse effects indicated. Supplemental. (██████, 6/23/97).

## STUDIES ON IMIDACLOPRID METABOLITES

### WAK3839

#### Rat Subchronic Dietary Toxicity

51950-0024 119520; “WAK 3839, Subchronic Toxicological Study on Rats (Twelve-Week Administration in Drinking Water)” (Krötlinger, F., Bayer AG, Department of Toxicology, Wuppertal, Germany, Report No. 101949, 3/2/92); 821; WAK 3839 (imidacloprid metabolite); 0, 112, 339, 1105 ppm in drinking water to 15 rats/sex/dose for 12 weeks (for only 4 weeks for 5/sex/dose- interim sacrifice group); all rats survived treatment period; no treatment related clinical signs, changes in hematological parameters, or changes in blood chemistry levels observed; necropsy and histopathology revealed no treatment related lesions; NOEL (M/F) > 1105 ppm (106 mg/kg/day); Acceptable. (██████ 4/15/93)

#### Mutagenicity

51950-025; 119521; 842; mutagenicity; “WAK 3839; Reverse Mutation Assay (*Salmonella typhimurium* and *Escherichia coli*)”; author: M. Watanabe; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Hino Institute, Tox. Research Lab., Japan; 11/26/90; report #100668; WAK 3839, a metabolite of NTN 33893; 98.3% purity; *S. typhimurium* strains TA98, TA100, TA1535, & TA1537, and *E. coli* strain WP2/uvrA; doses (-/+ S9): 312.5, 625, 1250, 2500, & 5000 µg/plate; positive controls were successful; either no effect or very weak effects of test article on revertant frequency were observed; WAK 3839 is not mutagenic in these systems under the conditions tested; Acceptable. (██████, 4/26/93)

51950-025; 119522; 842; mutagenicity; “WAK 3839; Mutagenicity Study for the Detection of Induced Forward Mutations in the V79-HGPRT Assay In Vitro”; author: H. Lehn; Bayer AG, Department of Toxicology, Wuppertal, FRG; 8/15/89; report #100662; WAK 3839, a metabolite of NTN 33893; 98.9% purity; doses (based on solubility limit and cytotoxicity test): 500, 1000, 1500, 1750, & 2000 µg/ml for both -S9 trials and 1 of 2 +S9 trials; for the other +S9 trial the doses were 500, 750, 1000, 1250, 1500, & 1750 µg/ml; after plating 4 x 10<sup>6</sup> cells/250 ml flask, the cells were exposed to test article (-/+ S9 microsomes) for 5 hr followed by an “expression period” of exponential growth and subsequent replating under selective conditions (10 µg/ml 6-thioguanine) at 3 x 10<sup>5</sup> cells/100 mm dish; after 7 days the colonies were fixed and counted; duplicate exposure dishes were run, each dish generating 8 replicate dishes in the selection condition; test article did not induce 6-thioguanine resistance at any dose despite success of positive controls (-S9, ethyl methane sulfonate; +S9, DMBA); it is not mutagenic in this system under these conditions; Acceptable. (██████, 4/26/93)

51950-025; 119523; 842; mutagenicity; “WAK 3839; Mutagenicity Study for the Detection of Induced Forward Mutations in the CHO-HGPRT Assay In Vitro”; author: H. Lehn; Bayer AG, Department of Toxicology, Wuppertal, FRG; 2/22/89; report #100661; WAK 3839, a metabolite of NTN 33893; 94.3%

purity; doses (based on solubility limit and cytotoxicity test), -S9: 62.5, 125, 250, 500, 1000, & 2000 µg/ml; +S9: 500, 750, 1000, 1250, 1500, & 2000; after plating 4 x 10<sup>6</sup> cells/250 ml flask, the cells were exposed to test article (-/+ S9 microsomes) for 5 hr followed by an “expression period” of exponential growth and subsequent replating under selective conditions (10 µg/ml 6-thioguanine) at 3 x 10<sup>5</sup> cells/100 mm dish; after 7 days the colonies were fixed and counted; duplicate exposure dishes were run, each dish generating 8 replicate dishes in the selection condition; test article did not consistently induce 6-thioguanine resistance at any dose despite success of positive controls (-S9, ethyl methane sulfonate; +S9, DMBA); it is not mutagenic in this system under these conditions; Acceptable. (██████, 4/27/93)

### Chromosomal Aberration

51950-025; 119524; 843; structural chromosome aberration; “WAK 3839 or NTN37571; Micronucleus Test on the Mouse After Intraperitoneal Injection”; author: B. A. Herbold; Bayer AG, Department of Toxicology, Wuppertal, FRG; 10/3/89; report #100664; WAK 3839 (aka NTN 37571), a metabolite of NTN 33893; 98.9% purity; dose (based on pilot toxicity test): 0 & 50 mg/kg body wt., administered intraperitoneally as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide (sacrificed @ 24 hr only); animals sacrificed 24, 48, & 72 hr post dose, bone marrow erythroblasts isolated from femur; 5/sex per sacrifice group; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; Acceptable. (██████, 4/27/93)

51950-025; 119525; 843; structural chromosome aberration; “NTN 37571: Micronucleus Test on the [sic] Mice After I.P. Treatment; Pilot Study”; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/29/88; report #100679; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; 96.4% purity; doses: 0, 20, 40, & 80 mg/kg body wt., administered intraperitoneally as a suspension in DMSO:olive oil (1:10, 10 ml/kg); positive control: 4 mg/kg mitomycin C; animals sacrificed 30 hr post dose, bone marrow erythroblasts isolated from femur; 5 males/dose; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatic or normochromatic cells despite the success of the positive controls; no change in the polychromatic/normochromatic cell ratio; Unacceptable (no females were tested, only a single sampling time was tested, and no individual data were presented). (██████, 4/27/93)

51950-025; 119527; 843; structural chromosome aberration; “WAK 3839; Micronucleus Test on the Mouse After Oral Application”; author: B. A. Herbold; Bayer AG, Department of Toxicology, Wuppertal, FRG; 10/3/89; report #100663; WAK 3839, a metabolite of NTN 33893; 98.9% purity; dose (based on pilot toxicity test): 100 mg/kg body wt., administered by gavage as a suspension in 0.5% aqueous Cremophor (10 ml/kg); positive control: 20 mg/kg cyclophosphamide; animals sacrificed 24, 48, & 72 hr post dose, bone marrow erythroblasts isolated from femur; 5/sex per sacrifice group; the 48-hr sacrifice group showed a statistically significant increase over controls in micronucleated polychromatics (2.0/1000 % 0.8 vs. 0.7/1000 % 0.9 in controls sacrificed at 24 hr, p<0.01) which may be partially accounted for by the abnormally low value of the controls compared to historical controls; slight non-statistically significant increases over negative controls were also observed in the # of micronucleated polychromatic cells in the 24- and 72-hr sacrifice groups; positive controls sacrificed at 24 hr raised the # of micronucleated cells to 16.1 % 7.9 per 1000 polychromatics; there may be a weak effect of the test article on micronucleus formation under these conditions; Acceptable. (██████, 4/27/93)



51950-025; 119528; 843; structural chromosome aberration; "NTN 37571: Micronucleus Test on the [sic] Mice After Oral Treatment; Pilot Study"; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/29/88; report #100680; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; 96.4% purity; doses (based on a preliminary toxicity determination): 0, 40, 80, & 160 mg/kg body wt., administered by gavage as a suspension in DMSO:polyethylene glycol 400 (1:5, 10 ml/kg); positive control: 4 mg/kg mitomycin C, injected intraperitoneally; animals sacrificed 30 hr post dose, bone marrow erythroblasts isolated from femur; 5 males/dose; no test article-induced increase over negative controls was observed in the # of micronucleated polychromatics or change in the polychromatic/normochromatic cell ratio despite the success of the positive controls in raising the # of micronucleated polychromatics and lowering the polychromatic/normochromatic ratio; Unacceptable (no females were tested, positive controls were not administered by the same route as the test article, only a single sampling time was used, and no individual data were provided). (██████, 4/28/93)

51950-025; 119529; 843; structural chromosome aberration; "Chromosome Aberration Assay in Chinese Hamster V79 Cells In Vitro with WAK 3839"; author: A. Heidemann; Cytotest Cell Research GmbH & Co., Robdorf, FRG; 9/27/89; report #100666; 98.8% purity; doses (based on a preliminary cytotoxicity determination and test article solubility), +/- S9: 0.1, 0.3, & 1.0 mg/ml; cultures harvested 7 (high dose only), 18, & 28 (high dose only) hr after start of the 4 hr exposure; positive controls ethyl methane sulfonate (-S9) and cyclophosphamide (+S9) showed distinct increases in aberrations; despite cytotoxicity of the test article at the mid and high dose indicated by a decline in mitotic index and at the high dose by a decline in plating efficiency (-S9 only), there was no increase in chromosome aberrations; WAK 3839 is not clastogenic in this system under these conditions; Acceptable. (██████, 4/28/93)

51950-025; 119530; 843; structural chromosome aberration; "NTN 37571: In Vitro Cytogenetic Assay Measuring Chromosome Aberrations in CHO-K1 Cells"; author: M. Usami; Nihon Tokushu Noyaku Seizo K.K., Basic Research Division, Agricultural Chemicals Institute, Japan; 11/5/88; report #100678; NTN 37571 (aka WAK 3839), a metabolite of NTN 33893; purity not reported; doses, +/- S9 (based on preliminary toxicity tests): 0, 0.25, 0.5 & 1 mg/ml; positive controls: -S9, 1 µg/ml N-methyl-N'-nitro-N-nitrosoguanidine; +S9, 0.5 mg/ml dimethylnitrosamine; exposure time: -S9, 24 & 48 hr; +S9, 4 hr; 4 x 10<sup>3</sup> cells/flask seeded (flask size not given), duplicate cultures exposed/condition, test article exposure began 48 hr later; colchicine added 2 hr prior to harvest to arrest cells in metaphase; 50 metaphases examined/flask (100/condition total); possible slight increase in % cells with chromosome aberrations under -S9 condition (control cells @ 48 hr w/aberrations excluding gaps = 1%, exposed cells = 2, 5, & 4%, respectively), but beneath the 10% limit considered by the investigators to be biologically relevant; no increase in +S9 cells; positive controls were successful; Unacceptable, but may be upgradeable upon submission of test article purity and size of flask used in assay. (██████, 4/29/93)

51950-025; 119531; 844; other genotoxic effects; "Unscheduled DNA Synthesis [UDS] in Primary Hepatocytes of Male Rats In Vitro with WAK 3839"; author: R. Fautz; 4/24/89; report #100665; 98.9% purity; hepatocytes (derived freshly from male animals because female-derived cells purportedly lack certain activating enzymes) seeded at 10<sup>5</sup>/ml in 35 mm culture dishes containing 1-25 mm cover slip; doses (based on a preliminary cytotoxicity determination and test article solubility), Expt. I & II: .04, .13, .44, 1.33, 4.44, 13.33, 44.44, 133.33, 444.44, & 1333.33 µg/ml (last 2 doses Expt. II only); Expt. III: 13.33, 44.44, 133.33, 444.44, & 1333.33 µg/ml; 18 hr exposure; triplicate dishes run at each concentration; positive control: 11.16 µg/ml 2-acetyl aminofluorene; UDS measured by autoradiographic

determination of <sup>3</sup>H-thymidine incorporation into DNA; severe cytotoxicity observed only in Expt. I above 133.33 µg/ml (other experiments were negative); no reproducible test article dependent increase in incorporation was observed under any condition despite the consistent success of the positive control; test article does not induce UDS in this system under the conditions tested; Unacceptable, but possibly upgradeable with submission of cytotoxicity data. (██████, 4/29/93)

### DATA GAP STATUS TABLE

<b>Study Type</b>	<b>Data Gap Status</b>
Combined Toxicity, Rat	No data gap, possible oncogenic effect
Chronic Toxicity, Dog	No data gap, no significantly toxic effects
Oncogenicity, Mouse	No data gap, no significantly toxic effects
Reproduction, Rat	No data gap, no significantly toxic effects
Developmental Toxicity, Rat	No data gap, no significantly toxic effect
Developmental Toxicity, Rabbit	No data gap, no significantly toxic effects
Gene Mutation	No data gap, no significantly toxic effects
Chromosome Effects	No data gap, possible significantly clastogenic effect
DNA Damage	No data gap, no significantly toxic effect
Neurotoxicity	No data gap, possible acutely toxic effect