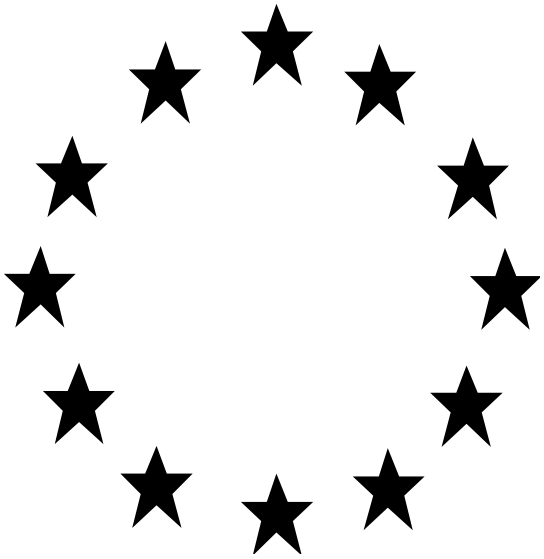


Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Germany for the existing active substance

IMIDACLOPRID

of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

Volume 3, Annex B, B.6

February 2006

Annex B

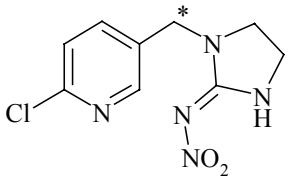
Imidacloprid

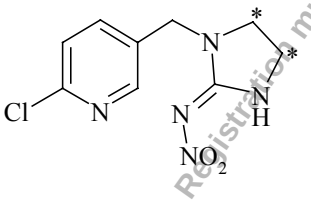
B-6: Toxicology and metabolism

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

The toxicokinetic and metabolic behaviour of the insecticidal active ingredient imidacloprid (NTN 33893) was investigated in the rat in various studies according to the US-EPA Pesticide Assessment Guidelines, the OECD Guideline and the guideline of the Japanese registration authority (Klein 1987a, Klein 1987b, Klein 1990, Klein and Karl 1990; Karl and Klein 1992, Klein and Brauner, 1991). These studies will be described in detail below. The test compounds used were labelled with [^{14}C] either in the methylene bridge or in the 4- and 5-positions of the imidazolidine ring:





* = position of label

In this section the investigations concerning toxicokinetics and metabolism in rats are summarised. Although study authors have used different names or codes for the degradation products of imidacloprid in these studies, a single name and a single code number for each metabolite are always used in this summary. The structures, different names, short forms and code numbers of all rat metabolites used in the study reports are provided in a separate list of metabolites.

B.6.1.1 Absorption, distribution and excretion of [pyridinyl-^{14}C-methylene]-imidacloprid

Report: Klein, O. (1987a)
[^{14}C]-NTN 33893: Biokinetic part of the "General metabolism study" in the rat.
Bayer AG, unpublished report No. PF2889, date: 1987-11-09

GLP: yes (certified laboratory)

Guideline: EPA Pesticide Assessment Guidelines, Subdivision F, EPA 54019-82-025 (November 1982) incl. the tissue distribution study to conform to the Japanese requirements.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: [^{14}C]-Labelled: [pyridinyl-^{14}-c-methylene]-imidacloprid, specific radioactivity 5.6 MBq/mg, radiochemical purity 99 %, chromatographic purity > 99 %.
[^{13}C]-Labelled: [pyridinyl-^{13}C-methylene]-imidacloprid, atom [^{13}C]-purity > 99 %
Imidacloprid, unlabelled, batch and purity not reported

Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany), body weight approximately 200 g
Number of test animals: 50 males, 20 females in groups of at least 5 animals.
During excretion studies animals were kept in cages which allowed a separate and quantitative sampling of the excreta. In all other cases animals were kept in plastic cages on wood shavings. The animals were kept at room temperature during the test period of 48 hours. In the non-radioactive pre-treatment period and during the bile cannulation the rats were housed under conditions of controlled temperature (20 °C) and humidity (40 - 80 %). Altromin 1324 standard food, 15 g per day and animal and water, ad libitum were provided.
The animals were sacrificed using carbon dioxide gas.

The collected blood was separated into plasma and erythrocytes by centrifugation. Organs and tissues collected during the experiment were weighed immediately after dissection and again following lyophilisation. Finally, they were homogenised before aliquots were taken for the determination of radioactivity by the combustion technique.

Table B.6.1-1: Rat ADME studies – Experimental design

Characteristics of experiment	Excretion with expired air	Excretion with bile, urine, faeces	Excretion with urine and faeces, plasma levels organ concentrations					
Remarks		bile-cannulated group	intravenous group		low dose group		high dose group	
Dose	20 mg/kg	1 mg/kg	1 mg/kg		1 mg/kg		20 mg/kg	
Route of administration	oral	intraduodenal	intravenous		oral		oral	
Sex	male	male	male	female	male	female	male	female
Number of animals	5	5	5	5	5	5	5	5
Duration of experiment	48 hrs.	48 hrs.	48 hrs.		48 hrs.		48 hrs.	

Characteristics	Time-dependent characterisation of metabolites in organs and tissues
Dose	single dose, 20 mg/kg
Route of administration	oral
Number of animals and sex	20 males (5 males/time point)
Duration of experiment	0.67, 1.5, 3, 6 hrs.; 48 hr organ values taken from high dose group males (see above)

Characteristics	Multiple dosing experiment (14 + 1 days):
Dose	14 days 1 mg/kg bw nonradioactive as once per day, day 15 (24 hours after the last nonradioactive dose) 1 mg/kg bw of ^{14}C-labelled as
Route of administration	oral
Number of animals and sex	5 males, 5 females
Duration of experiment	48 hrs. after administration of ^{14}C-labelled as

For the preparation of the dosing solutions the amounts of labelled and unlabelled imidacloprid shown in Table B.6.1-2 were dissolved in physiological saline solution using an ultrasonic bath at 70 °C. Rats were administered volumes of 10 mL/kg body weight (bw) in oral and intravenous route experiments and 1 mL/kg bw for intraduodenal dosing as a single dose.

Table B.6.1-2: Rat ADME studies – Doses administered

Dose [mg/kg]	Application	Concentration [mg/mL]	^{14}C –labelled [mg]	^{13}C-labelled [mg]	unlabelled [mg]	Saline solution
Single dose and multiple dose experiments						
1	intraduodenal	1	1.5			15 mL
1	intravenous	0.1	1.5			15 mL
1	oral	0.1	1.5			15 mL
20	oral	2	1.5	14.3	14.3	15 mL
Time-dependent characterisation experiment						
20	oral	2	5		95	50 mL

Plasma samples were taken at 5, 10, 20, 40 mins. and 1, 1.5, 2, 3, 4, 6, 8, 24, 32 and 48 hrs. post application. Urine was sampled in intervals of 0 – 4, 4 – 8, 8 – 24, 24 – 32 and 32 - 48 hrs. and faeces in periods of 0 - 24 and 24 – 48 hrs. after dosage. For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in the trapped combustion gases was measured by LSC. Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC. Liquid samples were mixed with scintillation gel and measured by LSC.

Quantitative evaluation:
Calculation of relative concentrations:

$$\text{Relative concentration P} = \frac{\text{Radioactivity measured / grams of plasma or tissue}}{\text{Radioactivity administered / grams of body weight}}$$

Equivalent concentrations (radioactivity of metabolites calculated as equivalents of the active substance) were calculated from relative concentrations by multiplying with the dose in mg per kg.

Amounts of radioactivity present in the excreta or still present at time of sacrifice in the tissues of the animal body or in the organs were calculated from measured concentrations and the weight contribution to the total body weight. Values were determined by weighing where possible. Values not accessible by weighing were estimated as follows:

Plasma: 3.2 %, erythrocytes 3.2 %, dissectable fat: 5.0 %, muscle: 40.0 %

Evaluation of kinetics:
Series of concentration-time data pairs were fitted to a sum of exponentials using the following equation:

$$x_i = \mathrm{sum}_{j=1}^{N}\left[A_{(j)} \times e^{\left(-b_{(j)} \times t_{(i)}\right)}\right]$$

where
A(j) ≡ preexponential factor weighting the proportion j^{th} exponential term in the fit function.
b(j) ≡ exponent of the j^{th} exponential term inversely proportional to the j^{th} half-life.
N ≡ number of exponential terms in the fit function corresponding to the number of phases in the experimental curve.

(For time courses of the level of concentration or amount the time axis is shifted by the lagtime Tlag which is the interval between administration and the onset of absorption.)

Findings:
Absorption
After oral administration of both, the high and low dose of [pyridinyl-^{14}C methylene] imidacloprid the maximum dose-normalised concentration of radioactivity in the plasma was reached between 1.1 and 2.5 hours. In all cases the peak concentration was low with an average of 0.73 mg/L, compared to the equidistribution of 1. Since the majority of the administered radioactivity was excreted renally, the absorption was assumed to be high. From the experiment using bile-cannulated rats and intraduodenal administration the amount of absorbed radioactivity was calculated to be 95 % of the given dose. This is in good agreement with the estimations for the oral tests. In all dose groups under investigation the rate of absorption can be described with an average half-life of approximately 35 minutes taking into account a lagtime of less than 2.5 minutes.

Distribution
After intravenous injection of 1 mg/kg bw, an apparent initial distribution volume (Vc) of about 84 % of the total body volume was obtained from plasma curve analysis for males and females. This result indicated that the radioactivity was readily distributed from the plasma into peripheral compartments. The distribution volume under steady-state conditions (Vss) was roughly in the same order of magnitude as the apparent initial distribution volume (Vc) after intravenous administration with the exception of male rats, receiving a single oral dose of 1 mg/kg bw. This supports the assumption that the radioactivity was distributed very quickly into peripheral compartments. It also means that the parent compound and/or its labelled metabolites have a high ability to permeate the tissues.

The Mean Residence Time (MRT) of the total radioactivity in the central compartment (plasma) varied between about 9 and 17 hours indicating that the redistribution into the plasma prior to elimination, mainly via the kidney, was also a fast process.

The radioactivity remaining in the body (excluding the gastrointestinal tract) at sacrifice 48 hours after oral or intravenous administration was below 1 % of the recovered radioactivity in all dose groups. However, from the kinetics of the renal excretion and of the elimination behaviour of the total radioactivity from the plasma it can be concluded, that the remaining radioactivity in the body was subject to further elimination. At the end of the experiment (48 h post application) the average dose-normalised concentration in the body (excluding gastrointestinal tract) was about 0.005 mg/L independent of the route of administration. Most of the investigated organs and tissues showed lower values. The highest

value was found in the kidney and the lowest value was detected in the brain [see Table B.6.1-3].
Identical patterns of distribution of total radioactivity were found in organs and tissues sampled at different times (40 min - 6 hours) following a single oral administration of 20 mg/kg bw. In this test maximum concentrations in all organs had been reached already 40 minutes after application [see Table B.6.1-4].

In summary it was found that the radioactivity administered with imidacloprid was very rapidly absorbed from the intestinal lumen and also readily distributed from the plasma into the body tissues.

Table B.6.1-3: Dose-normalised concentrations in tissues and organs at sacrifice after oral and intravenous applications of [pyridinyl-^{14}c-methylene] imidacloprid

Dose [mg/kg bw]	**1.0**		**1.0**		**1.0**		**20.0**	
Route of administration	**Single Intravenous**		**Single Oral**		**Multiple Oral (pre-treatment)**		**Single Oral**	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
Erythrocytes	0.0034	0.0037	0.0025	0.0033	0.0043	0.0037	0.0031	0.0032
Plasma	0.0051	0.0054	0.0046	0.0054	0.0066	0.0063	0.0047	0.0056
Spleen	0.0048	0.0045	0.0037	0.0029	0.0052	0.0036	0.0042	0.0036
GIT (see below)	0.0026	0.0055	0.0033	0.0044	0.0058	0.0116	0.0034	0.0038
Liver	0.0085	0.0115	0.0070	0.0088	0.0103	0.0106	0.0090	0.0094
Kidney	0.0110	0.0148	0.0086	0.0128	0.0223	0.0159	0.0128	0.0134
Testis	0.0016		0.0012		0.0017		0.0014	
Ovaries		0.0036		0.0047		0.0039		0.0030
Uterus		0.0043		0.0036		0.0042		0.0049
Muscle		0.0019	0.0017	0.0018	0.0020	0.0018	0.0020	0.0018
Bone	0.0026	0.0024	0.0023	0.0022	0.0024	0.0021	0.0020	0.0023
Heart	0.0030	0.0032	0.0022	0.0026	0.0045	0.0035	0.0034	0.0027
Lung	0.0110	0.0071	0.0094	0.0075	0.0133	0.0083	0.0127	0.0084
Brain	0.0009	0.0008	0.0006	0.0009	0.0008	0.0010	0.0009	0.0007
Skin	0.0096	0.0081	0.0093	0.0071	0.0150	0.0088	0.0156	0.0086
Residual carcass	0.0032	0.0027	0.0037	0.0029	0.0034	0.0049	0.0038	0.0027
Renal fat	0.0024	**0.0122**	0.0028	0.0027	0.0027	0.0015	0.0025	0.0013
Body excl. GIT	0.0055	0.0044	0.0051	0.0042	0.0069	0.0060	0.0068	0.0045

GIT = Gastrointestinal tract

Table B.6.1-4: Dose-normalised concentrations in male tissues and organs following a single oral dose of 20 mg/kg at various time points after dosage

	0.67 h	**1.5 h**	**3 h**	**6 h**	**48 h**
Spleen	0.7448	0.5872	0.5190	0.2406	0.0042
GI-Tract	3.8894	4.2312	2.3971	2.4652	0.0034
Liver	1.6094	1.3930	1.1822	0.5789	0.0090
Kidney	1.7000	1.5975	1.3470	0.7533	0.0128
Testis	0.4127	0.4554	0.4534	0.2172	0.0014
Muscle	0.6701	0.6034	0.5284	0.2392	0.0020
Heart	0.7499	0.6724	0.5845	0.2788	0.0034
Lung	0.9744	0.6805	0.5665	0.2960	0.0127
Skin	0.4066	0.2987	0.2604	0.1027	0.0156
Carcass	0.5538	0.5126	0.4811	0.2602	0.0038
Renal fat	0.6332	0.6045	0.5273	0.2731	0.0025
Plasma	0.5412	0.1584	0.0907	0.0365	0.0047
Body excl. GIT	0.6531	0.5939	0.5213	0.2704	0.0068

Each series consisted of five animals.

Excretion

In all tests of this study the elimination of the total radioactivity from the plasma could be approximated by a combination of two exponential terms from which elimination half-lives were calculated. These half-lives varied between ca. 2.6 to 3.6 and 26 to 118 hours, respectively.

The radioactivity was readily eliminated from the body. Within 48 h after administration about 92 % of an intravenous dose of 1 mg/kg bw and about 96 % of an oral dose were excreted via urine and faeces. The major part of the radioactivity was excreted via the kidneys [average ratio: 4 : 1 (urine : faeces)]. There were no differences between female and male rats. More than 90 % of the radioactivity found in urine was excreted within 24 hours after dosing, as can be expected from the fast distribution and redistribution of the radioactivity and the good water solubility of the parent compound and its metabolites. On average, the residual radioactivity in the body excluding the gastrointestinal tract at sacrifice was about 0.5 % and in the gastrointestinal tract about 0.06 % of the dose.
Bile-cannulated rats excreted only 4.7 % of the dose with the faeces, 56.4 % in the urine and about 36 % with the bile. The biliary excretion was very rapid. More than 90 % of the biliary radioactivity was already excreted after 12 hours. The course of elimination can be described by two exponential terms with half-lives of 2.9 and 10.1 hours, respectively.

The difference observed in renal excretion between bile-cannulated and 'intact' animals (57.5 versus 77.8 % of the recovered radioactivity) is a strong hint towards the existence of an enterohepatic circulation. A major part of the material reabsorbed from the gastrointestinal tract after biliary excretion appears to be eliminated via the kidney. The investigation of the expired air for radioactive CO_2 over a period of 48 hours did not reveal significant amounts of radioactivity. This demonstrates that the chosen labelling position within the molecule was stable with respect to the formation of volatile C-1-fragments. The results are summarised in Table B.6.1-5.

Table B.6.1-5: Excretion of total radioactivity and radioactive residues in the rat 48 h after application of [methylene-^{14}C]-imidacloprid (values are given in % of the administered radioactivity)

	Excretion and residues [% of the administered radioactivity]									
Route of administration	**i.v.**		**p.o.**		**p.o.**		**p.o.**		**p.o.**	**i.d.**
Dose [mg/kg bw]	**1.0**		**1.0**		**1.0 pre-treatment**		**20.0**		**20.0 CO_2 test**	**1.0 bile-test**
Sex	male	female	male	female	male	female	male	female	male	male
Bile										35.85
Urine	73.43	72.53	72.57	72.42	69.04	71.83	73.26	79.50	79.32	56.40
Faeces	19.34	17.45	20.26	25.45	23.83	22.74	21.25	17.14	24.72	4.69
GIT	0.029	0.048	0.034	0.045	0.067	0.128	0.032	0.043	0.062	0.107
Body excl. GIT	0.489	0.402	0.453	0.372	0.609	0.531	0.614	0.396	0.549	0.997
Recovery	93.29	90.43	93.32	98.29	93.55	95.23	95.16	97.08	104.69	98.04

GIT = Gastrointestinal tract

Conclusion:
Imidacloprid is rapidly and extensively absorbed from the gastrointestinal tract following oral administration to rats. Absorbed material is distributed to all organs and tissues, with the exception of brain, in concentrations similar to or higher than the concentrations measured in plasma. The major proportion of the radioactivity is excreted renally, either directly or after enterohepatic circulation. Excretion is nearly complete after 48 hours. There was no evidence for accumulation in any of the tissues.

Report: Klein, O. (1987b)
[^{14}C]-NTN 33893: Investigations on the distribution of the total radioactivity in the rat by whole body autoradiography.
Bayer AG, unpublished report No. PF2891, date: 1987-10-12

GLP: Not applicable

Guideline: Not applicable

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: [^{14}C]-Labelled: [pyridinyl-^{14}C-methylene]-imidacloprid, specific radioactivity 5.58 MBq/mg, radiochemical purity 99 %, chromatographic purity > 99 %.
[^{13}C]-Labelled: [pyridinyl-^{13}C-methylene]-imidacloprid, atom [^{13}C]-purity > 99 %
Imidacloprid, unlabelled
Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany), body weight approximately 200 g
Number of test animals: 7 males; 1 animal per time point
Animals were kept in cages which allowed a separate and quantitative sampling of the excreta. Altromin 1324 standard food, 18 g per day and animal, and water, ad libitum, were provided.

For the preparation of the administration solutions the amounts of labelled and unlabelled imidacloprid shown in Table B.6.1-6 were dissolved in physiological saline solution using an ultrasonic bath at 70 °C. The administration volume was 10 mL/kg body weight.

Table B.6.1-6: Rat tissue distribution – Doses administered

Dose [mg/kg]	Application	Concentration [mg/mL]	^{14}C –Labelled [mg]	^{13}C -Labelled [mg]	unlabelled [mg]	Saline solution
20.0	oral	2.0	5	12.5	12.5	15 mL
20.0	intravenous	2.0	1	2.5	2.5	3 mL

Following oral administration animals were sacrificed after 1, 4, 8, 24 and 48 hours using carbon dioxide gas. The animal given an intravenous dose was killed 5 minutes after injection. After sacrifice the animals were fixed in a stretched position using a metal template and immediately frozen at -70 °C. The template was removed and the frozen body embedded in a slurry of carboxymethylcellulose on the platform of a microtome. Sections of 50 µm thickness were prepared and attached to adhesive tape, freeze-dried overnight and exposed to an X-ray film at -20 °C. Times of exposure varied between 7 and 120 days.

Findings:
The radioactivity was readily absorbed and immediately distributed to the tissues and organs of the rat. The pattern of distribution demonstrated the high ability of the radioactivity to permeate the tissues: With the exception of the fatty tissues, the central nervous system, and the mineral part of the bones blackenings on the autoradiogrammes over all other parts of the body were observed 5 minutes after intravenous injection as well as 1 hour following oral gavage.

Higher concentrations were visible at later time points - except in the contents of the intestinal tract - in the endocrine glands, e.g. thyroid and adrenals. High concentrations were also seen in the walls of the aorta and in the connective tissue of the skin. After 24 hours all other organs and tissues displayed only very little amounts of radioactivity. The strong labelling of the kidney during the first 24 hours is a reflection of the high rate of the renal excretion of the administered radioactivity.

The concentration in the fatty tissues was very low during the whole investigation period; also only small amounts of radioactivity were observed in the central nervous system. This is in good agreement with the low degree of lipophilicity of the parent compound and its metabolites and simultaneously shows that the radioactivity does not pass the blood-brain barrier very easily.

With increasing time after administration the concentration of the radioactivity decreased in the organs and tissues. The relative pattern of distribution, i.e. the difference in concentration between different tissues, changed only slightly during the investigation period.

Conclusion:
The study confirms the results described in the previous study report (PF2889) with respect to distribution and excretion patterns.

B.6.1.2 Absorption, distribution and excretion of [imidazolidine-4,5-^{14}C]-imidacloprid

(The following report No. PF3629 by Klein and Brauner is also presented in chapter 6.1.4.2 dealing with the metabolisation of imidacloprid in rats. The summary given below deals only with the biokinetic aspects investigated in the study.)

Report: Klein, O. and Brauner, A. (1991)
[imidazolidine-4,5-^{14}C]-imidacloprid: Investigation of the biokinetic behaviour and metabolism in the rat.
Bayer AG, unpublished report No. PF3629, date: 1991-01-11

GLP: Yes (certified laboratory)

Guideline: EPA Pesticide Assessment Guidelines, Subdivision F, EPA 54019-82-025 (November 1982)

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: [^{14}C]-Labelled: [imidazolidine-4,5-^{14}C]-imidacloprid, specific radioactivity 4.6 MBq/mg, radiochemical purity 99 %, chromatographic purity >99 %. For the high-dose experiment labelled test substance was diluted with unlabelled imidacloprid (purity 99.8 %) which resulted in a specific activity of 0.031 MBq/mg.

Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany), body weight approximately 200 g
Number of test animals: 15 males, 5 females
During excretion studies animals were kept in cages which allowed a separate and quantitative sampling of the excreta. In all other cases animals were kept in plastic cages on wood shavings. The animals were kept at room temperature during the test period of 48 h. Altromin 1324 standard food, 15 g per day and animal, and tap water; ad libitum, were provided. The animals were sacrificed using carbon dioxide gas.

The collected blood was separated into plasma and erythrocytes by centrifugation. Organs and tissues prepared during the experiment were weighed immediately after dissection and again following lyophilisation. Finally, they were homogenised before taking aliquots for the determination of radioactivity by the combustion technique.

Imidacloprid dissolved in physiological saline or suspended in a 0.5 % tragacanth suspension was administered to groups of 5 animals in a volume of 10 mL/kg body weight. The following experiments were performed:

Table B.6.1-7: Rat ADME study – Experimental design

Characteristics of experiment	Collection of faeces, urine, carcass, expired air	Collection of faeces, urine, organs, time-dependent measurement of concentration in plasma		
Dose	1 mg/kg	1 mg/kg	1 mg/kg	150 mg/kg
Route of administration	oral	oral	oral	oral
Sex	male	male	female	male

For the preparation of the low dose administration solutions, 1.5 mg of labelled test substance was dissolved in 15 mL of physiological saline solution using an ultrasonic bath at 70 °C. For the high dose experiment, 1.5 mg of labelled test substance and 223.5 mg of unlabelled reference substance were suspended in 15 mL of 0.5 % of tragacanth suspension.
Plasma samples were taken at 5, 10, 20, 40 mins. and 1, 1.5, 2, 3, 4, 6, 8, 24, 32 and 48 hrs. post application. Urine and expired air were sampled in intervals of 0 - 2, 2 - 4, 4 - 8, 8 - 24, 24 - 32 and 32 - 48 hrs. and faeces in periods of 0 - 24 and 24 - 48 hrs. after dosage.

For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in the trapped combustion gases was measured by LSC. Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC. Liquid samples were mixed with scintillation gel and measured by LSC. For the description of the quantitative evaluation of results see above (Klein, 1987a).

Findings:
Absorption
The absorption based on the renal elimination was estimated to be more than 90 % of the given dose. A complete absorption of the administered radioactivity from the lumen of the gastrointestinal tract can be assumed when considering also the findings from the study using methylene-[^{14}C]-labelled imidacloprid. After oral administration of [imidazolidine-4,5-^{14}C]-imidacloprid, the maximum plasma concentration was reached between 1 hour (male rats, low dose), 1.5 hours (female rats, low dose) and 4 hours (male rats, high dose) after dosing. While the maximum dose-normalised concentrations are comparable between males (0.94) and females (0.89) treated with low dose, they are significantly lower in the plasma of male rats (0.39) after administration of the high dose. This is probably due to an incomplete and delayed absorption of the radioactivity from the lumen of the gastrointestinal tract after an oral dose of 150 mg/kg bw.

Distribution
The distribution of the total radioactivity from the plasma into peripheral compartments depended on the different dose levels. Elimination from the plasma proceeded with terminal half-lives between 9 hours (high dose) and 25 hours (low dose). The Mean Residence Time (MRT) in the central compartment (plasma) was 14.3 hours for the high dose rats and 8.8 hours for the low dose rats. The shorter half-life and the longer MRT in the high dose rats are an indication for a delayed and incomplete absorption of the test substance. The other pharmacokinetic parameters do not differ in a significant way between low and high dose groups. The total and the renal clearance demonstrate fast elimination of the total radioactivity from the plasma and hence from the body.

The [^{14}C]-labelled residues in the body at the end of the investigation period 48 hours after oral administration were at or below 1 % of the dose. However, from the kinetics of the renal excretion it can be concluded, that these amounts of radioactivity will be subject to further excretion. The average dose-normalised concentration in the plasma was calculated as 0.006 at sacrifice. Most of the investigated organs and tissues showed lower values. Concentrations similar to or higher than in plasma were measured in the kidney, the lung and the skin. The highest radioactivity concentrations were found in the liver, irrespective of the dose group. In general, the concentrations determined in organs and tissues of female rats were lower than those found in male rats. The results are summarised in Table B.6.1-8.

Table B.6.1-8: Dose-normalised concentrations of total radioactivity (mean values) in organs and tissues of male rats at sacrifice (48 h p. appl.) after oral application of [imidazolidine-4,5-^{14}C]-imidacloprid

	Relative concentration P of the total radioactivity		
Dosage	**1 mg/kg bw**	**150 mg/kg bw**	
Sex	**male**	**female**	**male**
Erythrocytes	0.0053	0.0047	0.0052
Plasma	0.0057	0.0061	0.0062
Spleen	0.0080	0.0062	0.0084
GIT	0.0054	0.0041	0.0129
Liver	0.0419	0.0180	0.0286
Kidney	0.0164	0.0102	0.0124
Fat	0.0118	0.0035	0.0087
Testis	0.0035	-	0.0030
Ovaries	-	0.0084	-
Uterus	-	0.0085	-
Muscle	0.0034	0.0026	0.0029
Bone	0.0045	0.0039	0.0056
Heart	0.0055	0.0044	0.0065
Lung	0.0226	0.138	0.0180
Brain	0.0010	0.0009	0.0010
Skin	0.0201	0.0112	0.0246
Carcass	0.0055	0.0041	0.0061

GIT = Gastrointestinal tract

Excretion

Excretion from the body was also a fast process: More than 90 % of the dose was renally excreted during the 48 h-test period. Approximately 75 % of the radioactivity was eliminated within the first 24 hours. The faecal elimination played a minor role; the residual radioactivity in the body at sacrifice amounted to maximally 1 % of the given dose. Very low amounts (0.1 %) of the administered radioactivity were found in the expired air. An overview on the radioactivity balance is given in Table B.6.1-9:

Table B.6.1-9: Excretion of total radioactivity and radioactive residues in the rat 48 h after oral application of [imidazolidine-4,5-^{14}C]-imidacloprid (values are given in % of the administered radioactivity)

	Excretion and residues [% of the administered radioactivity]			
Dosage	**1 mg/kg bw**		**150 mg/kg bw**	**1 mg/kg bw CO_2 test**
Sex	**male**	**female**	**male**	**male**
CO_2	-	-	-	0.111
Urine	89.88	93.79	90.69	88.20
Faeces	8.44	6.3	7.50	11.24
GIT	0.06	0.04	0.12	0.05
Body excl. GIT	0.94	0.059	1.02	0.84
Recovery	99.32	100.72	99.33	100.44

GIT = Gastrointestinal tract

Conclusion:
The biokinetic behaviour of [imidazolidine-4,5-^{14}C]-imidacloprid is very similar to the methylene-[^{14}C]-labelled compound. The administered radioactivity was rapidly and completely absorbed from the intestinal lumen and rapidly distributed in the body. The excretion was fast and the renal route of excretion (ca. 90 % on average) was even more predominant.

B.6.1.3 Special metabolism study with NTN33893-nitrosimine

The metabolite NTN33893-nitrosimine (M07) was identified as a minor constituent in edible plant commodities but not in the excreta of rats from short-term studies performed according to guidelines. Since nitroso-compounds may exert adverse effects, a study was considered necessary to compare imidacloprid and NTN33893-nitrosimine (WAK 3839, M07) with regard to excretion and metabolisation. Additionally, the in vivo formation of NTN33893-nitrosimine was investigated after a single, high oral dose of imidacloprid to non-pretreated rats and in rats and mice treated with a high dose of imidacloprid for one year.

Report: Klein, O. (1990)
Imidacloprid - WAK 3839: Comparison of biokinetic behaviour and metabolism in the rat following single oral dosage and investigation of the metabolism after chronic feeding of imidacloprid to rats and mice.
Bayer AG, unpublished report No. PF3432, date: 1990-07-17)

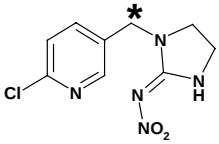
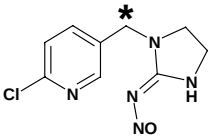
GLP: Yes (certified laboratory)

Guideline: Not given

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material:

[^{14}C-methylene]-imidacloprid	**[^{14}C-methylene]-NTN33893-nitrosimine (≡ [^{14}C-methylene]-WAK3839)**
	
*) position of label	
low dose experiments: Specific radioactivity 3.2 MBq/mg radiochemical purity > 99 % chemical purity > 99 % high dose experiments: Specific radioactivity 3.4 MBq/mg radiochemical purity > 99 % chemical purity > 99 % experiment with pre-treatment: Specific radioactivity 4.6 MBq/mg radiochemical purity 98.4 % content of NTN33893-nitrosimine 0.07 % chemical purity > 99 %	low dose experiments: Specific radioactivity 1.5 MBq/mg radiochemical purity 98.5 % chemical purity > 99 % isotope dilution experiment in rats: Specific radioactivity 1.87 MBq/mg radiochemical purity 98.2 % isotope dilution experiment in mice: Specific radioactivity 4.8 MBq/mg radiochemical purity 97.9 %

Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH & Co. KG, Germany), body weight approximately 200 g (single dose tests) and 380 g (pre-treatment study)
Number of test animals: 27 males
During excretion experiments animals were kept in cages which allowed a separate and quantitative sampling of the excreta. The animals were kept at room temperature during the test period of 72 hours. Altromin 1324 standard food, 15 g per day and animal and water, ad libitum, were provided. Animals in the pre-treatment experiment were fed over almost one year with a diet containing 1800 ppm imidacloprid ad libitum. At the end of the study the animals were sacrificed using carbon dioxide gas.

The collected blood was separated into plasma and erythrocytes by centrifugation. Organs and tissues prepared during the experiment were weighed immediately after dissection and again following lyophilisation. Finally, they were homogenised before aliquots were taken for the determination of radioactivity by the combustion technique.

Only for measurement of NTN33893-nitrosimine in urine: Mice from study "Carcinogenicity study in B6C3F1 Mice with administration in diet over a 24-month period", author Watta-Gebert, B; report No. 20769 (see B.6.5) chronically fed with a diet containing 2000 ppm of imidacloprid.

For administration the labelled and unlabelled compounds were either dissolved in physiological saline solution or suspended homogeneously in a 0.5 % tragacanth suspension. The dosages and amounts were as follows:

Table B.6.1-10: Rat ADME study with imidacloprid and NTN33893-nitrosimine – Doses administered

Compound	Dose [mL]	Dose [mg/kg]	No. of animals	Concen-tration [mg/mL]	Formulation	Amount [mg]	
						^{14}C	Unlabelled
Imidacloprid	2.0	1.0	5	0.1	saline	1.6	-
NTN33893-nitrosimine	2.0	1.0	5	0.1	saline	1.2	-
Imidacloprid	2.0	150	7	15.0	tragacanth	1.9	238
Imidacloprid	2.0	80 *)	10	15.0	tragacanth	3.0	372

*) considering a body weight of 380 g of the animals in the pretreatment group.

Low dose (imidacloprid and NTN33893-nitrosimine) and high dose (imidacloprid) groups:
Urine was sampled in intervals of 0 - 4, 4 - 8, 8 - 24 hrs. and 24 - 48 hrs. and faeces in periods of 0 - 24 and 24 - 48 hrs. after dosage.
In the pretreatment group urine was sampled in intervals of 0 - 4, 4 - 7, 7 - 24, 24 - 48 an 48 - 72 hours and faeces in periods of 0 - 24, 24 - 48 and 48 - 72 hours after administration. During the sampling period rats had free access to the diet containing 1800 ppm of imidacloprid.
All urine samples were cooled with dry ice and kept in the dark.
Plasma samples were taken at 5, 10, 20, 40 mins. and 1, 1.5, 2, 3, 4, 6, 8, 24, 32 and 48 hrs. post application.

For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in trapped combustion gases was measured by LSC.
Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC.
Liquid samples were mixed with scintillation gel and measured by LSC.

Evaluation of kinetics:
Series of concentration-time data pairs are fitted to a sum of exponentials using the following equation:

$$x_i = \mathrm{sum}_{j=1}^{N}\left[A_{(j)} \times e^{\left(-b_{(j)} \times t_{(i)}\right)}\right]$$

where
A(j)≡ preexponential factor weighting the proportion j^{th} exponential term in the fit function.
b(j) ≡ exponent of the j^{th} exponential term inversely proportional to the j^{th} half-life.
N ≡ number of exponential terms in the fit function corresponding to the number of phases in the experimental curve.

(For time courses of the level of concentration or amount the time axis is shifted by the lagtime Tlag which is the interval between administration and the onset of of absorption.)

After centrifugation the urine samples were directly injected for either preparative or analytical scale HPLC. The lyophilised and homogenised faeces of the first sampling interval (0 - 24 hrs.) were combined and homogenised per test group. Extraction was carried out repeatedly with water under ultrasonication. Extracts were filtered, combined and taken to dryness. The extracted solids were freeze-dried and homogenised in order to establish the radioactivity balance by radioactivity measurement. The reduced extracts were added to "Extrelut" resin and after the addition of a mixture of water-methanol (1+1) intensively homogenised under ultrasonication. The solvents were evaporated and the residue

exhaustively extracted successively with 1+1 mixtures of water–methanol and 2 % acetic acid-methanol. Further clean-up was achieved by preparative HPLC.

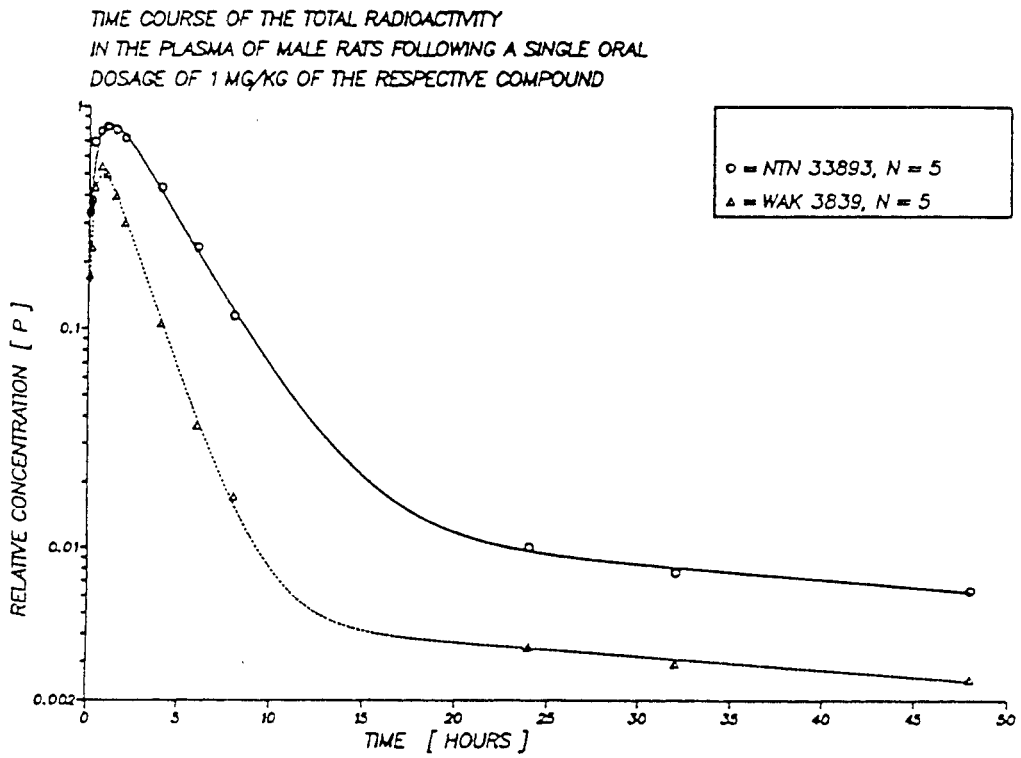
Concentrations of imidacloprid, NTN33893-nitrosimine (M07) and metabolites were determined by RP-HPLC using UV-diode array detector and a radioactivity flow-through detector. Metabolites were identified by mass spectroscopy in the direct inlet mode using the electron impact method or by 300 MHz H-NMR spectroscopy. NTN33893-nitrosimine was determined by direct isotope dilution analysis by comparison of the counts from the UV-signal and the radiochemical purity after addition of a definite amount of labelled analyte to the sample. Several purification steps were performed until the radiochemical purity reached a constant value.

Findings:

Absorption

The absorption of both test compounds started immediately after oral administration of 1 mg/kg bw without an apparent lag period. A comparison of the plasma curves suggested that the absorption was slightly lower for NTN33893-nitrosimine which may be related to the longer T_{max} observed for imidacloprid. Comparing the estimated half-lives of approximately 20 minutes for both compounds, the rate of absorption was similar in both cases. Maximal plasma concentrations were reached 1.16 hours after dosage of imidacloprid and 0.77 hours after administration of NTN33893-nitrosimine. The dose-normalised peak plasma concentration of imidacloprid (0.84) was higher in comparison to that of NTN33893-nitrosimine (0.51).

Figure B.6.1-1: Plasma time curves of imidacloprid and NTN33893-nitrosimine



Distribution

The distribution of the total radioactivity from the plasma into peripheral compartments could be approximated by a combination of two exponential terms, from which half-lives were calculated. The terminal half-life of NTN33893-nitrosimine was about 10 hours longer than that of imidacloprid (46.9 vs. 37.5 hours). Based on the significant difference of the basic pharmacokinetic parameters, total and renal clearance and the distribution volume at steady state also differ in a significant manner. The distribution volume at steady state is higher in the case of NTN33893-nitrosimine. The Mean Residence Time (MRT) was comparable for both compounds [Table B.6.1-11].

Table B.6.1-11: Pharmacokinetic parameters after oral dosage of 1 mg/kg of [methylene-^{14}C]-imidacloprid or [methylene-^{14}C]-NTN33893-nitrosimine

Parameter	Imidacloprid	NTN33893-nitrosimine
Pmax	0.84	0.51
Tmax (h)	1.16	0.77
Half-life, absorption. (h)	0.36	0.29
Half-life, elimination. (h)	35.7	46.9
CL (mL/min)	0.72	1.99
CL-R (mL/min)	0.55	1.46
MRT (h)	12.3	15.9
V-ss (L/kg)	2.82	9.44

The [^{14}C]-labelled residues in the body at the end of the investigation period 48 hours after oral administration were below 1 % of the given dose for both compounds and the kinetics of the renal excretion show that these small amounts of radioactivity will be subject to further excretion. After dosing of imidacloprid, the average dose-normalised concentration in plasma is calculated as 0.005 at sacrifice. Most of the investigated organs and tissues showed lower values than plasma. Higher concentrations were measured in the liver, the kidney, the lung and the skin. The highest radioactivity concentrations were found in the skin. In contrast, after dosing of NTN33893-nitrosimine the highest residue level was found in the heart. In addition, levels in the brain were increased as compared to the parent compound [Table B.6.1-12].

Table B.6.1-12: Dose-normalised concentration of total radioactivity (mean values) in organs and tissues of male rats at sacrifice (48 h p. appl.) after oral dosage of 1 mg/kg of [methylene-^{14}C]-imidacloprid or [methylene-^{14}C]-NTN33893-nitrosimine

	Imidacloprid	NTN33893-nitrosimine
Plasma	0.0051	0.0011
Erythrocytes	0.0033	0.0015
Spleen	0.0049	0.0021
GIT	0.0033	0.0037
Liver	**0.0087**	0.0028
Kidney	**0.0111**	0.0008
Testis	0.0013	0.0016
Muscle	0.0021	0.0014
Bone	0.0024	0.0014
Heart	0.0039	**0.0072**
Lung	**0.0125**	0.0003
Brain	0.0007	**0.0024**
Skin	**0.0250**	0.0023
Residual Carcass	0.0037	0.0056
Renal fat	0.0025	0.0011
Body excl. GIT*	0.0085	0.0020

*GIT = gastrointestinal tract

Excretion

As far as the excretion of the total radioactivity is concerned, no significant difference was observed between the two compounds. NTN33893-nitrosimine was eliminated slightly faster from the body. Excretion is almost complete within 48 hours with residues in the body amounting to less than 1 % of the given doses. Also, the excretion ratio between urine and faeces in both cases is approximately 3:1 for both compounds. After the administration of a single high dose of 150 mg/kg imidacloprid to male rats, the excretion ratio (urine to faeces) of the total radioactivity was the same as after low dose treatment. However, the rate of excretion was significantly reduced. Chronic pretreatment did not affect the excretory pattern [Table B.6.1-13].

Table B.6.1-13: Excretion of total radioactivity and radioactive residues in the rat at sacrifice following a single oral administration to male rats.

	Excretion and residues [% of the administered radioactivity]			
Test substance	**Imidacloprid**	**NTN33893-nitrosimine**	**Imidacloprid**	
Dosage	**1 mg/kg bw**		**150 mg/kg bw**	**80 mg/kg bw***
Test-No.:	**1**	**2**	**3**	**5**
Urine	77.3	72.7	74.2	80.2
Faeces	21.4	14.3	19.7	17.7
Gastrointestinal tract	0.04	0.02	1.33	
Body excl. GIT	0.85	0.20	2.09	
Recovery	99.6	87.2	97.3	97.9

* animals after one year of chronic feeding

Metabolism

The metabolic pattern in the excreta was investigated after administration of a single oral dose of 1 mg/kg of imidacloprid and NTN33893-nitrosimine to male rats and following the dosage of a high dose of 150 mg/kg imidacloprid to male rats. After the low dose of imidacloprid approximately 82 % of the renal radioactivity was identified, the main renal metabolite (about

30 %) was NTN33893-6-CNA-glycine (M15). Besides the parent compound (ca. 12.5 %) the two monohydroxylated biotransformation products, NTN33893-5-hydroxy and NTN33893-4-hydroxy (M01, M02, ca. 18.6 %) and NTN33893-olefine (M06, ca. 11.2 %) were found in greater amounts. NTN33893-6-CNA (6-chloronicotinic acid, M14) was quantified with 7.9 % of the radioactivity in urine. The formation of NTN33893-nitrosimine (M07) did not take place under these dosing conditions. In the faeces, which contained about. 25 % of the given dose, imidacloprid, NTN33893-olefine (M06), 6-chloronicotinic acid (M14) and its glycine conjugate (M15) were identified. These findings are in good agreement with those reported in the study of Klein and Karl, 1990 (see below).

In the urine of male rats, which were orally dosed with NTN33893-nitrosimine (M07), almost no metabolisation products were observed. In addition to the bulk radioactivity consisting of unchanged NTN33893-nitrosimine about 8 % of the radioactivity was due to NTN33893-desnitro (M09). There is some evidence that this metabolite is also present in the faeces. A summary of these metabolism results is provided in Table B.6.1-14.

Table B.6.1-14: Identified metabolites in the excreta of male rats 24 hours after administration of a single oral dose of 1 mg/kg bw imidacloprid and NTN33893-nitrosimine (% of total radioactivity, M07))

	Imidacloprid		**NTN33893-nitrosimine**
	Urine	**Faeces**	**Urine**
Imidacloprid	12.6	4.3	
NTN33893-6-CNA-glycine (M15)	31.6	1.1	
NTN33893-5-hydroxy (M01)	18.6		
NTN 35884 (M06)	11.2	2.4	
NTN33893-6-CNA (≡ 6-chloro-nicotinic acid, M14)	7.9	2.0	
NTN33893-nitrosimino (M07)	0.6		70.8
NTN 38014 (M09)			8.0
Sum identified	82.4	9.8	78.8

In order to examine the possibility for in vivo formation of NTN33893-nitrosimine (M07) as a biotransformation product of imidacloprid after high dose exposure a single oral administration of 150 mg/kg imidacloprid was given to male rats. However, under these conditions, no in vivo formation of NTN33893-nitrosimine was observed. The amount found in the urine was quantitatively identical to the trace impurity contained in the radioactive batch of the parent compound administered.

Further studies were conducted in rats after chronic ingestion of imidacloprid for one year at a dose level of 1800 ppm in the diet (approximately 100 mg/kg bw/d). These rats received a single tracer dose of [methylene-^{14}C]-imidacloprid by oral gavage before their urine was analysed for metabolites. In this test some 90 % of the total urinary radioactivity was excreted during the period of 7 to 24 h after administration of the tracer. The chromatographic analysis of the urine sample revealed that 9.31 % of the radioactivity was attributable to NTN33893-nitrosimine which corresponded to 6.85 % of the administered dose. The concentration in the urine was calculated to be ca. 8 mg/100mL. This shows that NTN33893-nitrosimine is being formed in vivo by rats under chronic feeding conditions. In addition to NTN33893-nitrosimine other metabolites identified already after a single low dose of imidacloprid were detected. In order to support these finding, a direct isotope dilution analysis was conducted in the urine of chronically pretreated rats (one year at 1800 ppm imidacloprid in the diet) and mice (one year at 2000 ppm imidacloprid in the diet). Both dilution analyses clearly

demonstrated the existence of NTN33893-nitrosimine in the urine of either species. The concentration in the urine of rats was determined to be ca. 9 mg/100 mL, this matched the result which was obtained after administration of a radioactive tracer dose. The corresponding result in chronically pretreated mice was ca. 1.5 mg/100 mL of urine.

These findings support the hypothesis that the reduction of the NO_2 moiety of imidacloprid required for formation of NTN33893-nitrosimine (WAK 3839, M07) is a biotransformation reaction in rats and mice which obviously only takes place if enzymes catalysing other possible reactions, e.g. the oxidative cleavage to 6-chloronicotinic acid (M14), are saturated by a chronic "flooding" of the liver with imidacloprid.

Conclusion:
Metabolism of imidacloprid in rats after low and high dose exposure was found to be very similar when animals were given a single oral dose. However, long-term exposure of rats and also mice may lead to the formation of additional metabolites from reduction of the NO_2 moiety. Despite the differences in metabolism between imidacloprid and NTN33893-nitrosimine their biokinetic behaviour with respect to overall retention and tissue exposure was very similar. Therefore, accumulation of NTN33893-nitrosimine in long-term studies is not expected.

B.6.1.4 Metabolism of imidacloprid in rats

A metabolism study of imidacloprid according to the EPA Pesticide Assessment Guidelines was performed with [methylene-^{14}C]-labelled compound (Klein and Karl, study report No. 3316). In a second metabolism study performed in accordance with guidelines of MAFF (Japan) the time dependence of distribution patterns of metabolites in some organs was investigated with methylene-labelled imidacloprid (Karl and Klein, PF3635). Since approximately 40 % of the identified metabolites in that metabolism study did not contain the imidazolidine moiety, a comparative study using a label in this heterocycle became necessary for a better understanding of the metabolic behaviour of imidacloprid in the rat (Klein and Brauner, PF3629; the study is also presented in chapter 6.1.2 dealing with the biokinetic behaviour of imidacloprid in rats. The summary of study PF3629 given below in chapter 6.1.4.2 deals only with those parts of the study in which the biochemical transformation processes were investigated).

B.6.1.4.1 Metabolism of [^{14}C-methylene]- imidacloprid

Report:	Klein, O. and Karl, W. (1990) Methylene-[^{14}C]-imidacloprid: Metabolism part of the General Metabolism Study in the rat. Bayer AG, unpublished report No. PF3316, date: 1990-01-30
GLP:	Yes (certified laboratory)
Guideline:	EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and domestic animals, Series 85-1: General Rat Metabolism Study, EPA 540/9-82-025 (November 1982)

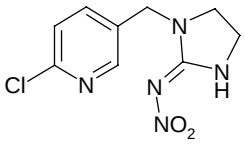
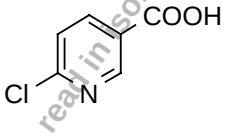
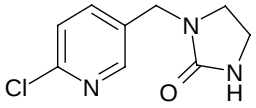
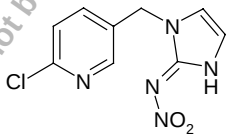
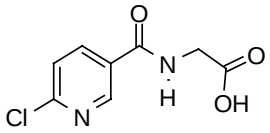
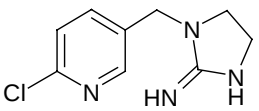
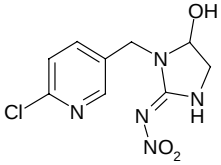
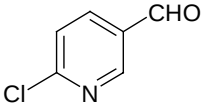
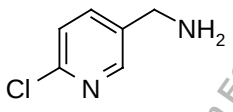
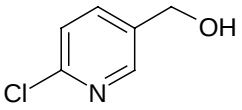
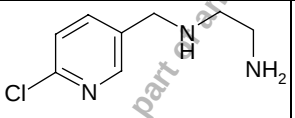
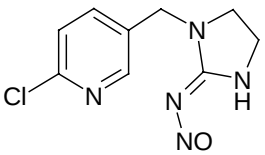
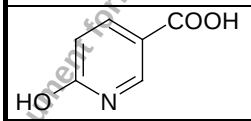
Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany), body weight approximately 200 g
The rats which produced biological samples (urine and faeces) were identical with those from study PF2889 (Klein) and study PF3635 (Klein and Karl).

Test material: [^{14}C]-Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 5.6 MBq/mg, radiochemical purity > 99 %
[^{13}C]-labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 5.6 MBq/mg, [^{13}C]-atom purity > 99 %, chemical purity > 98.5 %
Non-labelled imidacloprid, chemical purity 99.9 %
For identification of metabolites the following reference substances were used:

Table B.6.1-15: Rat metabolism study – Reference substances

Structural formula	Report names and codes	Structural formula	Report names and codes
	NTN33893 **as**		NTN33893-6-CNA **M14**
	NTN33893-urea **M12**		NTN33893-olefine **M06** NTN 35884
	NTN33893-6-CNA-glycine **M15**		NTN33893-desnitro **M09**
	NTN33893-5-hydroxy **M01** WAK4103		MAT 10249
	NTN33893-AMCP **M16**		NTN33893-CHMP **M28**
	NTN33893-PEDA **M22**		NTN33893-nitrosimine **M07** WAK3839
	6-Hydroxy-nicotinic acid **M18**		

Imidacloprid was administered in physiological saline solutions at different doses to groups of at least 5 animals.

Table B.6.1-16: Rat metabolism study – Experimental design

Characteristics of experiment	Excretion with expired air	Excretion with bile, urine, faeces	Excretion with urine and faeces, plasma levels organ concentrations					
Remarks	-	bile-cannulated group	intravenous group		low dose group		high dose group	
Dose	20 mg/kg	1 mg/kg	1 mg/kg		1 mg/kg		20 mg/kg	
Route of administration	oral	intraduodenal	intravenous		oral		oral	
Sex	male	male	male	female	male	female	male	female
Number of animals	5	5	5	5	5	5	5	5
Duration of experiment	48 hrs.	48 hrs.	48 hrs.		48 hrs.		48 hrs.	

Characteristics	Multiple dosing experiment (14 + 1 days):
Dose	14 days 1 mg/kg bw nonradioactive as once per day, day 15 (24 hours after the last nonradioactive dose) 1 mg/kg bw of ^{14}C-labelled as
Route of administration	oral
Number of animals and sex	5 males, 5 females
Duration of experiment	48 hrs. after administration of ^{14}C-labelled as

For the preparation of the administration solutions the amounts of labelled and unlabelled imidacloprid shown in Table B.6.1-17 were dissolved in physiological saline solution using an ultrasonic bath at 70 °C.

Table B.6.1-17: Rat metabolism study - Doses administered

Dose [mg/kg]	**Application**	**Concentration [mg/mL]**	**^{14}C –labelled [mg]**	**^{13}C-labelled [mg]**	**unlabelled [mg]**	**Saline solution**
1	intraduodenal	1	1.5	-	-	15 mL
1	intravenous	0.1	1.5	-	-	15 mL
1	oral	0.1	1.5	-	-	15 mL
20	oral	2	1.5	14.3	14.3	15 mL

Volumes of 10 mL/kg body weight were administered for orally and intravenously while 1 mL/kg body weight was given intraduodenally as a single dose.

Urine and faeces samples were taken from study No. PF2889 (Klein and Brauner). In some cases also urine samples from study PF3635 (Karl and Klein) were used for elucidation of metabolite structures. Urine was collected separately for each rat under cooling in intervals of 0 - 4, 4 - 8, 8 - 24 and 24 - 48 hours and faeces were collected in intervals of 0 - 24 and 24 - 48 hours postdose.

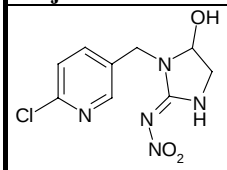
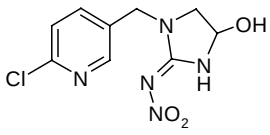
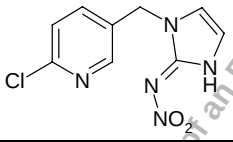
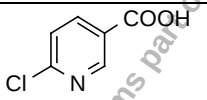
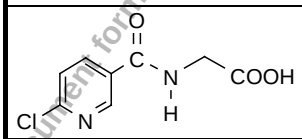
In some cases urine samples were directly injected for HPLC-investigations. For the separation of the NTN33893-5-hydroxy and - 4-hydroxy the 24 h-urine samples from the high dose female group were purified by means of adsorption chromatography on an “Extrelut” column. The lyophilised and homogenised faeces (0 - 24 hrs.) were combined per dose group and sex. Extraction was carried out repeatedly with water under ultrasonication. Extracts were filtered, combined and reduced to 50 mL. Extracted solids were freeze-dried and

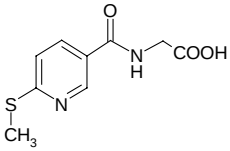
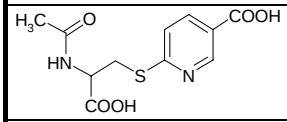
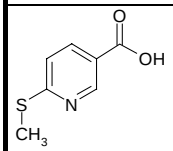
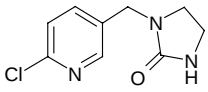
homogenised in order to establish the radioactivity balance by radioactivity measurement. Precipitates were removed by centrifugation. The clear supernatant was mixed with hydrochlorid acid and sodium chloride solution and added to "Extrelut" resin. Exhaustive extraction of radioactivity from the resin was achieved by rinsing successively with a 1 + 1 mixture of ethylacetate and butanol, with butanol and finally with a mixture 40 + 1 of butanol and concentrated hydrochloric acid. Phases were assayed for radioactivity and and appropriate ones were combined after the neutralisation of the hydrochloric acid. Solutions were reduced to dryness, reconstituted in a mixture of 1 mL of water and 0.2 mL of methanol and centrifuged.

Isolation and purification of metabolites for structural elucidation:
The identification was conducted either by chromatographic comparison with authentic reference compounds in at least two independent chromatographic systems or by 1H-NMR- and mass-spectroscopic techniques. Urine samples were separated by semi-prep HPLC on RP-silica-gel. In some cases samples were derivatised with diazomethane in methanol.

Findings:
The metabolism of [pyridinyl-^{14}C-methylene]-imidacloprid in the rat was investigated in the excreta of the animals of the biokinetic investigations. In addition to the unchanged parent compound, the metabolites presented in Table B.6.1-18 were identified by means of the techniques indicated. The quantitative distribution as percent of the recovered radioactivity of imidacloprid and the identified metabolites in the excreta (sum of urine and faeces) in rats 24 h after administration is given in Table B.6.1-19.

Table B.6.1-18: Metabolites isolated and identified in rat metabolism study

	Report name and code	Isolation and structural elucidation
as	imidacloprid	prep. RP-HPLC; H-NMR; mass spectroscopy
Major metabolites:		
	NTN33893-5-hydroxy **M01**	prep. RP-HPLC; H-NMR; mass spectroscopy comparison with reference substance by HPLC
	NTN33893-4-hydroxy **M02**	prep. RP-HPLC; H-NMR; mass spectroscopy separation between metabolite M01 and M02 by means of HPLC on amino phases
	NTN33893-olefine **M06**	prep. RP-HPLC; H-NMR; mass spectroscopy only from analogue urine sample; EI was identical except from the isotopic pattern caused by the ^{13}C/^{14}C-ratio.
	NTN33893-6-CNA **M14**	prep. RP-HPLC; H-NMR; mass spectroscopy comparison with reference substance by HPLC
	NTN33893-6-CNA-glycine **M15**	prep. RP-HPLC; derivatisation with diazomethane; H-NMR; mass spectroscopy comparison of the derivatised molecule with that of reference substance EI-mass spectroscopy

	Report name and code	Isolation and structural elucidation
Minor metabolites:		
	NTN33893-6-methylmercapto-nicotinic acid-glycine **M19**	prep. RP-HPLC; DCI- mass spectroscopy; H-NMR derivatisation with diazomethane; high resolution-mass spectroscopy of derivatised form
	NTN33893-mercapturic acid –NA **M21**	prep. RP-HPLC; derivatisation with diazomethane; GC-MS–spectroscopy of derivatised form
	NTN33893-6-methylmercapto-nicotinic acid **M20**	prep. RP-HPLC; EI-MS derivatisation with diazomethane; GC-MS–spectroscopy of derivatised form
	NTN33893-urea **M12**	prep. RP-HPLC; GC-MS–spectroscopy of derivatised form comparison with reference substance by MS

After administration of the low imidacloprid dose to rats there was only very little sex dependence in the excretion pattern and in the metabolic profiles of the excreta. In contrast, after oral administration of the high dose, some dependence on gender was detected both in the excretion pattern - females showed a somewhat higher renal elimination rate than males - and in the metabolic profile. Male animals showed a higher tendency to metabolise the test compound with an increased formation of NTN33893-olefine; the amount of unchanged parent compound was significantly lower than in females. The formation of other biotransformation products was not affected by the high dose in either sex. The average identification rate of metabolites in the excreta of all dose groups was approximately 78 % of the recovered radioactivity. All identified metabolites were found in each dose group and both sexes, with any differences being quantitative rather than qualitative in nature. The main metabolites include 6-chloronicotinic acid (M14) and its glycine conjugate (M15) which were only found in urine. The monohydroxylated metabolites, NTN33893-5-hydroxy and NTN33893-4-hydroxy were detected at similar concentrations as the unchanged parent compound. All other biotransformation products were quantitatively of minor significance.

Table B.6.1-19: Quantification of the metabolites in the excreta (sum of urine and faeces in % of the recovered radioactivity) following administration of [pyridinyl-^{14}C-methylene]-imidacloprid

Administration Route	**i.v.**		**p.o.**		**p.o.**		**p.o.**	
Dose	**1 mg/kg**		**1 mg/kg**		**15 x 1 mg/kg**		**20 mg/kg**	
Sex	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
NTN33893-6-CNA (M14)	7.6	5.6	4.3	3.2	7.0	5.9	7.2	8.2
NTN33893-6-methylmercapto-nicotinic acid-glycine (M19)	4.1	6.5	4.7	7.0	6.0	7.3	4.9	4.3
NTN33893-6-CNA-glycine (M15)	25.9	21.7	28.1	24.1	16.6	18.9	23.6	24.2
NTN33893-olefine (M06)	10.4	9.6	11.0	10.0	13.8	10.3	14.9	8.7
NTN33893-5-hydroxy (M01)[*)]	16.0	16.3	16.9	14.8	18.2	15.0	17.3	16.0
NTN33893-desnitro (M09)	2.6	2.4	2.3	2.4	3.4	3.0	2.2	2.2
Imidacloprid	15.3	17.0	13.4	13.2	12.0	14.0	9.8	15.9
Totally identified	81.9	79.0	80.8	74.8	77.1	74.4	80.0	79.4

*) Fraction included NTN33893-4-hydroxy

Conclusion:
Metabolic transformations of imidacloprid in the rat, can be distinguished into two main pathways: Oxidative cleavage of the parent molecule yields 6-chloronicotinic acid, which then reacts with glycine to form a hippuric acid-type of conjugate. Both metabolites are excreted quickly and exclusively via urine and represent the major part of the identified metabolites in the excreta, i.e. ca. 30 % of the recovered radioactivity. Less significant in terms of quantity is the dechlorination of the pyridine moiety yielding 6-hydroxy-nicotinic acid and its mercapturic acid derivative, probably as a degradation product of the glutathione conjugate. However, the possibility cannot be excluded, that glutathione is reacted directly with 6-chloronicotinic acid to form the same metabolite. The same holds true for the 6-methylmercaptonicotinic acid. Quantitatively the glycine conjugate of this metabolite is somewhat more important, as it is excreted via urine and faeces at an average rate of ca. 5.6 % of the recovered radioactivity. The second major route of biotransformation is initiated by the hydroxylation of the imidazolidine ring in either the 4- or the 5-position. Elimination of H_2O yields the "olefinic" metabolite NTN 35884. These biotransformation products are excreted via urine and faeces, together with significant amounts of the parent compound. The guanidine-type reduction product (NTN 33823) was only eliminated with the faeces. In terms of quantity this product is of minor importance.

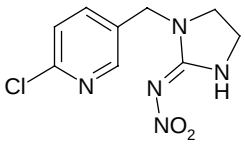
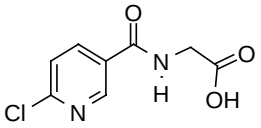
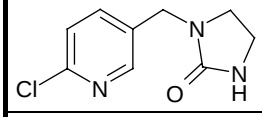
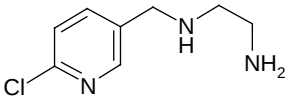
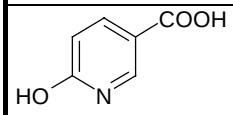
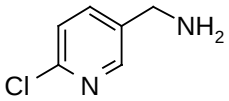
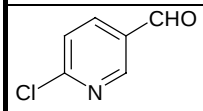
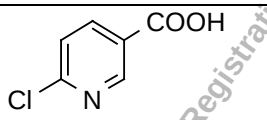
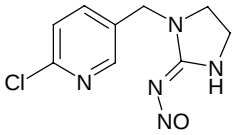
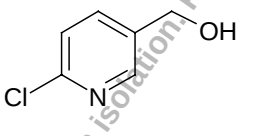
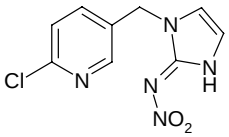
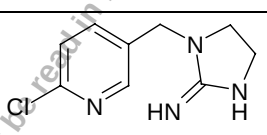
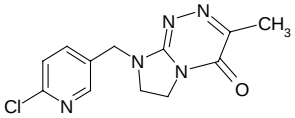
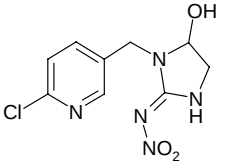
Report:	Karl, W. and Klein, O. (1992): [Pyridinyl-^{14}C-methylene]-imidacloprid: Distribution of the metabolites in some organs at different times following single oral administration to rats. Bayer AG, unpublished report No. PF 3635, date 1992-03-12
GLP:	Yes (certified laboratory**)**
Guideline:	Guidelines of the Japanese registration authorities (MAFF); special request
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: [^{14}C]-Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 5.6 MBq/mg, radiochemical purity > 99 %
Non-labelled imidacloprid, chemical purity 99.9 %

Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstierzucht GmbH & Co. KG, Germany), body weight approximately 200 g
The rats which produced biological samples were identical with those from study PF2889 (Klein) and study PF3316 (Klein and Karl).

For identification of metabolites the following reference substances were used:

Table B.6.1-20: Organ distribution study of metabolites in rats – Reference substances

Structural formula	Report names and codes	Structural formula	Report names and codes
	NTN33893 **as**		NTN33893-6-CNA-glycine **M15**
	NTN33893-urea **M12**		NTN33893-PEDA **M22**
	6-Hydroxy-nicotinic acid **M18**		NTN33893-AMCP **M16**
	MAT 10249		NTN33893-6-CNA **M14**
	NTN33893-nitrosimine **M07** WAK3839		NTN33893-CHMP **M28**
	NTN33893-olefine **M06** NTN 35884		NTN33893-desnitro **M09**
	NTN33893-triazinone **M25**		NTN33893-5-hydroxy **M01** WAK4103

Imidacloprid was administered p.o. in one single dose of 20 mg/kg in 10 mL/kg body weight to a group of 25 male rats. For the preparation of the administration solutions the amounts of labelled and unlabelled imidacloprid shown in the table below were dissolved in physiological saline solution using an ultrasonic bath at 70 °C.

Table B.6.1-21: Organ distribution study of metabolites in rats - Doses administered

Dose [mg/kg]	Application	Concentration	^{14}C –Labelled imidacloprid	unlabelled imidacloprid	saline solution
20	oral	2 mg/mL	5 mg	95 mg	50 mL

The biological material taken for the quantitative analyses consisted of the combined samples of liver and kidney, respectively from five animals at four different times of sacrifice (0.67, 1.5, 3, 6, 48 hrs postdose).

The time-dependent formation of metabolites from imidacloprid was investigated in liver and kidney of rats. The metabolites were extracted from the lyophilised organs with water and methanol and further purified by HPLC and TLC. The balances of the extraction steps were checked by radioactivity measurements. The identification was conducted by comparative

HPLC with authentic reference compounds in at least two independent chromatographic systems and also by mass- and 1H-NMR spectroscopic techniques.

Findings:
The radioactivity contents of the different organs calculated in µg of imidacloprid-equivalents per g of tissue are listed in Table B.6.1-22.

Table B.6.1-22: Equivalent concentrations in the organs and tissues of male rats following a single oral dose of 20 mg/kg

	Concentrations [µg as equivalents /g] found for different times of sacrifice			
	0.67 h	**1.5 h**	**3 h**	**6 h**
Spleen	14.90	11.74	10.38	4.81
Gastrointestinal tract	77.79	84.62	47.94	49.30
Liver	32.19	27.86	23.64	11.58
Kidney	34.00	31.95	26.94	15.07
Testis	8.25	9.11	9.07	4.34
Muscle	13.40	12.07	10.57	4.78
Heart	15.00	13.45	11.69	5.58
Lung	19.49	13.61	11.33	5.92
Skin	8.13	5.97	5.21	2.05
Residual carcass	11.08	10.25	9.62	5.20
Renal fat	12.66	12.09	10.55	5.46
Plasma	10.82	3.17	1.81	0.73
Body (excl. GIT)	13.06	11.88	10.43	5.41

Five animals were sacrificed at each timepoint**.**

The residues detected in liver and kidney were extracted and analysed. The extraction yields were approximately 95 % for the kidneys and 70 % for the livers. The concentrations of the identified metabolites in µg parent compound equivalents per gram of fresh weight are summarised in Table B.6.1-23.

The metabolites found in the kidney are identical with those identified in the urine (Klein, O. and Karl, W. 1990). However, NTN33893-triazinone (M25) produced in the liver was not found in the excreta. Its formation can be explained by a non-enzymic condensation of a hypothetical NTN33893-amino-guanidine intermediate (WAK 3877/4, M08) with pyruvic acid. This reaction was also applied in synthesising the reference compound. The triazinone derivative probably underwent further biodegradation prior to elimination via the kidney or the bile. The relative amounts of NTN33893-6-CNA (M14) in the liver formed by oxidative mechanisms at the labelled methylene bridge remained relatively constant in the first 6 hours, possibly due to a steady state between elimination and continuing formation from precursors, e.g. NTN33893-desnitro (M09). In the kidney the excretory behaviour of this particular organ governs the metabolite pattern inasmuch as the relative amount of the more polar compounds decreases with time (NTN33893-6-CNA (M14), NTN33893-6-CNA-glycine (M15)) while NTN33893-olefine (M06) and NTN33893-5-hydroxy (M01) show a relative increase at the expense of the parent compound.

Table B.6.1-23: Concentration (µg/g fresh weight) of the metabolites in liver and kidney after a single oral dose of [pyridinyl-^{14}C-methylene]-imidacloprid to male rats

	Liver				Kidney			
Time post application (h)	**0.67**	**1.5**	**3**	**6**	**0.67**	**1.5**	**3**	**6**
NTN33893-6-CNA (M14)	0.7	0.7	0.6	0.6	2.4	3.2	1.9	1.0
NTN33893-6-CNA-glycine(M15)	-	-	-	-	4.6	3.7	3.4	2.0
NTN 33893-urea (M12)	1.5	1.0	0.8	0.3	-	-	-	-
NTN33893-triazinone (M25)	2.2	2.1	1.8	1.0	-	-	-	-
NTN33893-desnitro (M09)	7.5	6.0	5.9	2.2	-	-	-	-
NTN33893-olefine (M06)	-	-	-	-	1.8	2.0	2.1	1.4
NTN33893-5-hydroxy (M01)	-	-	-	-	1.0	0.9	1.1	1.2
Imidacloprid	-	-	-	-	17.1	15.4	15.0	7.5
Sum	11.9	9.8	9.1	4.1	26.9	25.2	23.4	13.1
Total Residue	32.2	27.9	23.6	11.6	34.0	32.0	26.9	15.1

Conclusion:
While the metabolite pattern in the kidney is similar to the pattern found in urine, three additional (intermediate) metabolites (M09, M12, M25) have been identified in the liver.

B.6.1.4.2 Metabolism of [^{14}C-4,5-imidazolidine]-imidacloprid

Report: Klein, O. and Brauner, A. (1991)
[Imidazolidine-4,5-^{14}C]-imidacloprid:
Investigation of the biokinetic behaviour and metabolism in the rat.
Bayer AG, unpublished report No. PF3629, date: 1991-01-11

GLP: yes (certified laboratory)

Guideline: EPA Pesticide Assessment Guidelines, Subdivision F,
Hazard Evaluation: Human and domestic animals, Series 85-1: General Rat Metabolism Study EPA 540/9-82-025 (November 1982)

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: [^{14}C]-Labelled: [imidazolidine-4,5-^{14}C]-imidacloprid, specific radioactivity 4.6 MBq/mg, radiochemical purity 99 %, chromatographic purity > 99 %.
For the high-dose experiment tabelled test substance was diluted with unlabelled reference substance of imidacloprid (purity 99.8 %) which resulted in a specific activity of 0.031 MBq / mg.

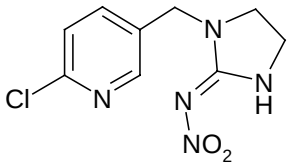
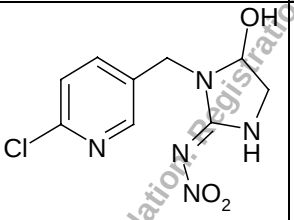
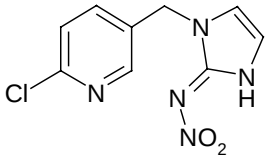
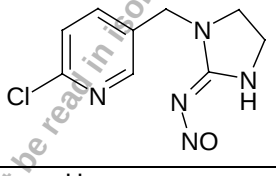
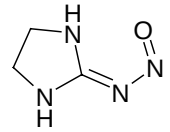
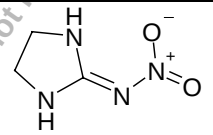
Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann Versuchstier-zucht GmbH&Co. KG, Germany), body weight approximately 200 g
Number of test animals: 15 males, 5 females
During excretion studies animals were kept in cages which allowed a separate and quantitative sampling of the excreta. In all other cases animals were kept in plastic cages on wood shavings. The animals were kept at room temperature during the test period. Altromin

1324 standard food, 15 g per day and animal and tap water, ad libitum, were provided. The animals were sacrificed using carbon dioxide gas.

The collected blood was separated into plasma and erythrocytes by centrifugation. Organs and tissues prepared during the experiment were weighed immediately after dissection and again following lyophilisation. Finally, they were homogenised before taking aliquots for the determination of radioactivity by the combustion technique.

For identification of metabolites the following reference substances were used:

Table B.6.1-24: Metabolism of [^{14}C-4,5-imidazolidine]-imidacloprid – Reference substances

Structural formula	Report names and codes	Structural formula	Report names and codes
	NTN33893 as		NTN33893-5-hydroxy M01 WAK4103
	NTN33893-olefine M06 NTN 35884		NTN33893-nitrosimine M07 WAK3839
	Nitrosoimino imidazolidine WAK5080		Nitroimino imidazolidine M26 NTN33968

Imidacloprid was administered either dissolved in physiological saline or suspended in a 0.5 % tragacanth suspension groups of 5 animals (10 mL/kg body weight). The following experiments were performed:

Table B.6.1-25: Metabolism of [^{14}C-4,5-imidazolidine]-imidacloprid – Experimental design

Characteristics of experiment	Collection of faeces, urine, carcass, expired air,	Collection of faeces, urine, organs, time dependent measurement of concentration in plasma,		
Remarks	-	-	-	-
Dose	1 mg/kg	1 mg/kg	1 mg/kg	150 mg/kg
Route of administration	oral	oral	oral	-
Sex	male	male	female	male

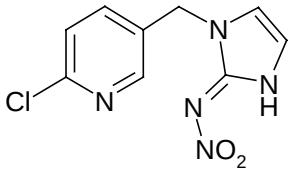
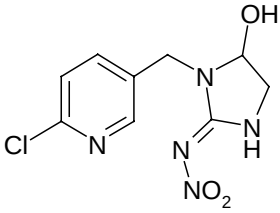
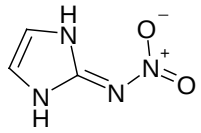
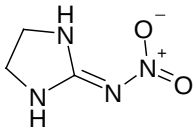
For the preparation of the low dose solutions 1.5 mg of labelled test substance was dissolved in 15 mL of physiological saline solution using an ultrasonic bath at 70 °C. In the case of the high dose experiment 1.5 mg of labelled test substance and 223.5 mg of unlabelled reference substance were suspended in 15 mL of 0.5 % tragacanth suspension.

The urine of the dose groups 1 mg/kg (male and female) and 150 mg/kg (male) was analysed for metabolites. For structure elucidation purposes the urine collected from the high dose group within 24 hrs. after application was pooled (385 mL), lyophilised and redissolved in water. The solution was adjusted to pH 2 and purified on XAD4 resin and charcoal. Adsorption on silica gel followed by step-wise solid phase extraction with solvent mixtures of rising polarity resulted in fractions containing the major part of the radioactivity. These fractions were concentrated and subjected to semi-preparative scale TLC on silca gel plates of 2 mm thickness. Two peak groups appeared which were further fractionated by analytical scale HPLC. Homogeneous single peaks were collected and further analysed by H-NMR and mass spectroscopy. No attempts were made to extract metabolites from faeces since it they contained only between 6.3 and 8.5 % of the administered dose.

Analysis and structure elucidation were performed using RP-HPLC with UV-spectrophotometer and flow-through radioactivity detector. Identification of peak components using reference substances took place with mass spectroscopy in direct inlet mode and H-NMR, 300 MHz.

Findings:
In addition to the unchanged parent compound the following metabolites were identified (Table B.6.1-26). The metabolites M26 and M27 consisting only of the imidazolidine ring system could not be detected in previous studies performed with the methylene-labelled test substance.

Table B.6.1-26: Metabolism of [^{14}C-4,5-imidazolidine]-imidacloprid – identified metabolites

Structural formula	Report names and codes	Structural formula	Report names and codes
	NTN33893-olefine M06 NTN 35884		NTN33893-5-hydroxy M01 WAK4103
	Nitroimino dehydro-imida-zolidine M27		Nitroimino imidazolidine M26 NTN33968

These metabolites were found in the urine which contained approximately 90 % of the administered radioactivity, corresponding to an identification rate of 77 % of the recovered radioactivity. The quantitative distribution of the parent compound and the identified metabolites is given in Table B.6.1-27.

Table B.6.1-27: Quantification (% applied radioactivity) of the metabolites in urine after single oral administration of [4,5-^{14}C-imidazolidine] imidacloprid to rats

Dose (mg/kg)		**1.0**	**1.0**	**150**
Report name	**M-No.**	**Male**	**Female**	**Male**
Nitroimino-dehydro-imidazolidine	M27	34.7	29.6	19.1
Nitroimino imidazolidine	M26	8.0	15.7	18.4
NTN33893-olefine	M06	14.7	13.7	14.6
NTN33893-5-hydroxy	M01	8.4	7.7	9.1
Imidacloprid		6.9	16.5	14.2
Totally identified		72.7	83.2	75.4

Conclusion:
The findings are compatible with the proposed two main metabolic pathways in the rat, oxidative cleavage of the parent compound and hydroxylation of the imidazolidine ring in either the 4- or the 5-position.

B.6.1.4.3 Summary of the biokinetics and metabolism of imidacloprid in rats

The biokinetic studies in rats showed that imidacloprid is rapidly and almost completely absorbed from the intestinal lumen. Also the elimination from the organism is fast and complete, there is no indication of any bioaccumulation potential of the parent compound and/or its metabolites. Both, absorption and elimination progress independently from the route of administration. On average, three quarters of the administered radioactivity is excreted with the urine, the remainder is found in the faeces. Most of the faecal radioactivity originates from biliary excretion.

Peak plasma concentrations are reached within approximately 1-2 hours. The radioactivity is rapidly distributed from the intravascular space to the peripheral tissues and organs. At 48 h after dose application radioactivity in the tissues are very low. Levels above average are only observed in the contents of the gastrointestinal tract, liver, kidney, adrenals, thyroid, connective tissues and the vascular walls of the aorta. The extent of penetration of the blood-brain barrier is very limited.

The metabolisation rate of imidacloprid in the rat is very high and is somewhat more pronounced in male than in female animals. The amount of unchanged parent compound in excreta varied between 10 and 16 % of the given dose. The main renally excreted metabolites are 6-chloronicotinic acid (M14) and its glycine conjugate (M15) as well as the two corresponding imidazolidine ring-containing biotransformation products M26 and M27. The two monohydroxylated metabolites NTN33893-5-hydroxy (M01) and NTN33893-4-hydroxy (M02) as well as NTN33893-olefine (M06) are also detected in the urine. The latter is also excreted with the faeces together with NTN33893-6-CNA (M14) and NTN33893-6-CNA-glycine (M15).

Studies on the biokinetic and metabolic behaviour of imidacloprid and its nitrosimino plant metabolite NTN33893-nitrosimine (≡ WAK 3839 ≡ M07) in male rats yielded comparable data for absorption, distribution and elimination. However, NTN33893-nitrosimine was eliminated somewhat more rapidly, and the radioactivity levels in the organs were lower as compared to imidacloprid. No NTN33893-nitrosimine was detected in the urine or faeces

following administration of single oral doses of 1 mg/kg bw and 150 mg/kg bw of imidacloprid to male rats. After high doses of imidacloprid had been given to rats and mice in the diet over a one-year period, NTN33893-nitrosimine was found in the urine at levels of 9 mg/100 mL (rat) and 1.5 mg/100 mL (mouse), respectively. Reduction of the nitro group of imidacloprid leading to the formation NTN33893-nitrosimine apparently only takes place in chronic exposure situations with high imidacloprid concentrations which are likely to saturate the enzyme systems catalysing other possible degradation reactions. The toxicological properties of NTN33893-nitrosimine thus may influence the results of the chronic toxicity studies in the rat and mouse (cf. also Machemer, 1992).

Two major routes of metabolism responsible for the degradation of imidacloprid can be derived. The first involves an oxidative cleavage yielding nitroimino-imidazoline (M26) and 6-chloronicotinic acid (M14) which is conjugated with glycine to form M15. These metabolites were found only in the urine and were excreted very quickly. They constitute the major part of the identified metabolites, representing ca. 30 % of the recovered radioactivity. Only of minor importance in terms of quantity is the dechlorination of the pyridinyl moiety leading to the 6-hydroxy-nicotinic acid (M18) and its methylmercapto derivative (M20), probably as a degradation product of a glutathione conjugate. Possibly glutathione can also react directly with 6-chloronicotinic acid (M14) to form the same metabolite. The 6-methylmercapto nicotinic acid conjugated with glycine (M19) amounted to 5.6 % of the recovered radioactivity.

The second important biodegradation step starts with the hydroxylation of the imidazolidine ring in the 4- or 5-position. About 16 % of the recovered radioactivity was identified as the sum of 4- and 5-hydroxy-imidacloprid (M01, M02). The loss of water yields NTN33893-olefine (M06). These biotransformation products and the unchanged parent compound were excreted with urine and faeces, while NTN33893-desnitro (M09) as a less important metabolite was eliminated only with the faeces.

From the quantitative point of view the results with different positions of the ^{14}C-label are very similar. If one compares the formation of nitroimino-dehydroimidazolidine (M27) and nitroimino-imidazolidine (M26) on the one hand with 6-chloronicotinic acid (M14) its glycine conjugate (M15) and 6-methylmercaptonicotinic acid (M20) on the other hand, it can be seen that the percentage distribution in the urine is of the same order of magnitude (cf. Table B.6.1-28). It has to be kept in mind that the renal excretion of the total radioactivity is higher after dosing [imidazolidine-4,5-^{14}C]-imidacloprid. Biotransformation products of both heterocycles exhibit a very similar quantitative distribution pattern.

The high excretion rate of the parent compound (average of 14 %) indicates a quick passage through the body which is confirmed additionally by the elimination of more than 90 % of the recovered radioactivity within 24 hours after administration.

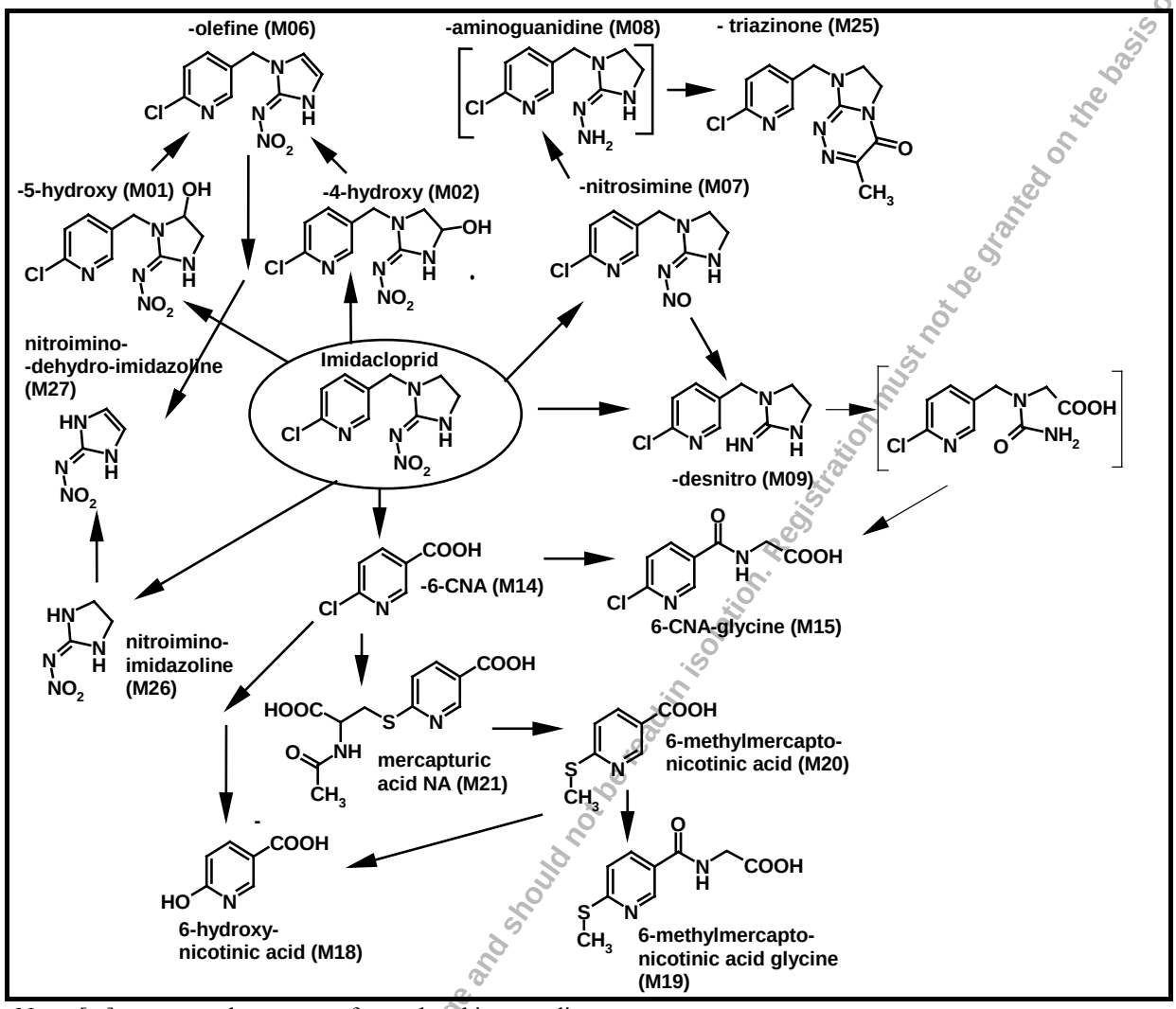
Table B.6.1-28: Metabolites found in the urine of rats treated orally with differently radio labelled imidacloprid; radioactivity accumulated in 48 hours after treatment (% of recovered radioactivity)

	[Imidazolidine-4, 5-^{14}C] NTN33893 1 mg/kg bw p.o.		[Methylene-^{14}C] NTN33893 1 mg/kg bw p.o.	
	male	**female**	**male**	**female**
Imidacloprid	6.9	16.5	11.3	11.3
Nitroimino-dehydroimidazolidine (M27)	34.7	29.6	-	-
NTN33893-6-CNA-glycine (M15)	-	-	28.1	24.1
NTN33893-5-hydroxy (M01)	14.7	13.7	16.9	14.8
NTN 33893-olefine (M06)	8.4	7.7	9.9	8.6
Nitroimino-imidazolidine (M26)	8.0	15.7		
6-Chloro-nicotinic acid (M14)	-	-	4.3	3.2
6-Methylmercapto-nicotinic acid (M20)	-	-	2.7	5.1
Total identified	72.7	83.2	73.2	67.1

B.6.1.4.4 Proposed metabolic pathway of imidacloprid in rats

The metabolites identified in the metabolism studies in rats can be arranged into a pathway indicating a sequence of reactions leading to their formation and further transformation. Oxydative cleavage of the methylen-bridge gives rise to chloronicotinic acid and nitroimino-imidazoline (Figure B.6.1-2). A second important sequence starts with the hydroxylation of the imidazoline ring system. Finally reduction of the nitro group occurs with formation of NTN33893-nitrosimine as a reactive intermediate which is rapidly transformed and or excreted.

Figure B.6.1-2: Proposed metabolic pathway of imidacloprid in the rat



Note: [...] = proposed structure of postulated intermediate

B.6.2 Acute toxicity including irritancy and skin sensitisation (Annex IIA 5.2)

Imidacloprid has a moderate acute toxicity after oral administration to rats and exhibited a higher acute oral toxicity in mice than in rats. When combining the data from three oral studies in the same rat strain LD_{50} values of 522 mg/kg bw and 506 mg/kg bw can be derived for males and females, respectively. Imidacloprid is non-toxic after acute dermal application as well as after acute inhalatory exposure.
Imidacloprid is neither an eye nor a skin irritant, and has no skin sensitising potential.

Table B.6.2-1: Summary of acute toxicity, primary irritation and sensitisation studies

Oral						
Species	Vehicle	Sex	NSD§ [mg/kg bw]	LLD& [mg/kg bw]	LD_{50} [mg/kg bw]	Reference
Rat	Cremophor/water 2 % v/v	male	50	400	~ 424	Bomann, 1989a
		female	100	400	450 < LD_{50} < 475	
		male	50	350	642	Bomann, 1991a
		female	100	450	648	
		male	50	300	504	Bomann, 1991b
		female	100	300	379	
Rat	Cremophor/water 2 % v/v	male	50	300	522	Data from the three studies combined*
		female	100	300	506	
Mouse	Cremophor/water 2 % v/v	male	10	100	131	Bomann, 1989b
		female	10	120	168	
Percutaneous						
Species	Vehicle/exposure	Sex	NSD [mg/kg bw]	LLD [mg/kg bw]	LD_{50} [mg/kg bw]	Reference
Rat	NaCl solution 0.9 % (24 h)	male	5000	not established	> 5000	Kroetlinger, 1989
		female	5000	not established	> 5000	
Inhalation						
Species	Exposure	Sex	NSC§ [mg/m³ air]	LLC& [mg/m³]	LC_{50} [mg/m³ air]	Reference
Rat	aerosol (4 h)	male	69	not established	> 69	Pauluhn, 1988a
		female	69	not established	> 69	
	dust (4 h)	male	1220	not established	> 5323	
		female	1220	not established	> 5323	

§ NSD / NSC: no symptom dose / concentration & LLD /LLC: lowest lethal dose / concentration
* calculated with U.S.EPA NCEA BenchMark Dose Software, version 1.3.2

B.6.2.1 Oral

Report: Bomann, W. (1989a)
NTN 33893 - Study for acute oral toxicity to rats.
Bayer AG, unpublished report No.: 18594, date: 15.12.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mixed batch 180587, purity: 94.2 %, was formulated in Cremophor® EL / demineralised water (2 % v/v). The test substance was administered in a single dose by oral gavage to fasted SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Application volume: 10 mL/kg bw.

Findings:

Table B.6.2-2: Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
Males				
50	0/0/5	-	-	0
100	0/5/5	40 m - 1 d	-	0
250	0/5/5	40 m - 1 d	-	0
315	0/5/5	20 m - 1 d	-	0
400	1/5/5	15 m - 2 d	3 h	20
450	4/5/5	25 m - 6 d	2 h - 1 d	80
500	5/5/5	20 m - 7 h	2 h - 7 h	100
1800	5/5/5	15 m - 3 h	1 h - 3 h	100
Females				
100	0/0/5	-	-	0
250	0/5/5	40 m - 1 d	-	0
315	0/5/5	15 m - 2 d	-	0
400	1/5/5	20 m - 2 d	6h	20
450	0/5/5	25 m - 2 d	-	0
475	5/5/5	30 m - 7 h	2 h - 7 h	100
500	5/5/5	40 m - 6 h	2 h - 6 h	100
1800	5/5/5	15 m - 1 d	2 h - 1 d	100
LD_{50} rat,	males: ~ 424 mg/kg bw females: 450 < LD_{50} < 475 mg/kg bw			

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Clinical signs: Apathy and labored breathing were the findings observed at a dose of 100 mg/kg bw; at higher doses, clinical signs additionally included accelerated breathing, decreased motility, staggering gait, narrowed eyelids, trembling and spasms.

Body weights: Body weight development may have been disturbed initially as documented by slight decrements in weight gain observable 4 days postdose in animals treated with 250-400 mg/kg body weight and higher.

Gross necropsy: In the animals which died during the post treatment period, the following findings were recorded: liver dark; spleen pale, slightly dark in one animal; lung dark, patchy and distended; glandular stomach mucosa slightly reddened. No test substance-related changes were noted in animals sacrificed at the end of the observation period.

Conclusion:
Imidacloprid is moderately toxic to rats following acute oral administration.

Report: Bomann, W. (1991a)
NTN 33893 AMP (proposed c.n.: imidacloprid) – Study for acute oral toxicity in rats.
Bayer AG, unpublished report No.: 20591, date: 3.9.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, batch no. 17133/90, purity 96.0 %, was formulated in Cremophor EL® / demineralised water (2 % v/v). Single oral doses of the test substance were administered by stomach tube to fasted SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Application volume: 10 mL/kg bw.

Findings:

Table B.6.2-3: Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
Males				
50	0/0/5	-	-	0
200	0/5/5	20 m - 1 d	-	0
350	1/5/5	55 m - 3 d	4 h	20
400	3/5/5	1 h - 4 d	4 h - 1 d	60
500	1/5/5	25 m - 4 d	7 h	20
600	0/5/5	15 m - 8 d		0
750	3/5/5	15 m - 3 d	5 h - 6 h	60
1000	5/5/5	45 m - 2 d	2 h - 2 d	100
Females				
100	0/0/5	-	-	0
400	0/5/5	1 h - 2 d	-	0
450	2/5/5	40 m - 4 d	3 h - 1 d	40
500	1/5/5	25 m - 4 d	2 h	20
600	2/5/5	15 m - 2 d	6 h - 7 h	40
1000	5/5/5	30 m - 6 h	4 h - 6 h	100
LD_{50} rat,	males: 642 mg/kg bw females: 648 mg/kg bw			

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Clinical signs: Apathy, staggering or spastic gait, labored breathing, transient or continuing spasms, transient tremor, decreased motility, increased water intake, diuresis, piloerection, salivation, absence of feces and transient convulsions.
Body weights: Body weight development may have been disturbed initially as documented by slight decrements in weight gain observable 4 days postdose in animals treated with 200-400 mg/kg body weight and higher.

Gross necropsy: The following findings were recorded in animals which died during the post-observation period: lungs distended, patchy, dark; liver dark; kidney slightly pale; bladder engorged with urine; spleen slightly pale. No test substance-related changes were noted in animals sacrificed at the end of the observation period.

Conclusion:
Imidacloprid is moderately toxic to rats following acute oral administration.

Report: Bomann, W. (1991b)
NTN 33893 CNS (c.n.: *imidacloprid* (proposed)) - Study for acute oral toxicity to rats.
Bayer AG, unpublished report No.: 20637, date: 20.9.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mixed batch 180587, purity: 94.3 %, was formulated in Cremophor® EL / demineralised water (2 % v/v). The test article was administered in a single dose by oral gavage to fasted SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Application volume: 10 mL/kg bw.

Findings:
Clinical signs: Apathy, staggering and spastic gait, labored breathing; at higher doses reduced motility, spasmodic state, periodic tremors, soft faeces and piloerection.
Body weight gain: Body weight development may have been disturbed initially as documented by slight decrements in weight gain observable 4 days postdose in animals treated with 300 - 350 mg/kg body weight and higher.

Table B.6.2-4: Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
Males				
50	0/0/5	-	-	0
200	0/5/5	20 m - 1 d	-	0
300	1/5/5	50 m - 2 d	5 h	20
350	1/5/5	55 m - 3 d	6 h	20
400	2/5/5	55 m - 5 d	1 d	40
500	1/5/5	25 m - 3 d	6 h	20
600	4/5/5	10 m - 5 d	2 h - 3 h	80
Females				
100	0/0/5	-	-	0
200	0/5/5	55 m - 7 h	-	0
300	1/5/5	50 m - 2 d	1 d	20
350	2/5/5	55 m - 3 d	4 h - 6 h	40
400	2/5/5	55 m - 3 d	4 h - 7 h	40
500	5/5/5	35 m - 1 d	2 h - 1 d	100
LD_{50} rat,	males: 504 mg/kg bw females: 379 mg/kg bw			

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Gross necropsy: Findings in animals that died during the post-treatment observation period included: lung distended, mottled, dark; liver dark; bladder distended with clear urine. No test article-related gross pathological findings were observed in the animals sacrificed at the end of the post-treatment observation period.

Conclusion:
Imidacloprid is moderately toxic to rats following acute oral administration.

Report: Bomann, W. (1989b)
NTN 33893 - Study for acute oral toxicity to mice.
Bayer AG, unpublished report No.: 18593, date: 15.12.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mix batch 180587, purity: 94.2 %, was formulated in Cremophor® EL / demineralised water (2 % v/v). The test substance was administered in a single dose by gavage to fasted SPF-bred mice (Strain Bor: NMRI; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Application volume: 10 mL/kg bw.

Findings:
Clinical signs: Apathy, labored breathing, decreased motility, transient staggering gait, transient trembling and transient spasms.
Body weights: No effects were observed on the body weight development.
Gross necropsy: The following findings were described for animals which died during the observation period: liver pale, occasionally dark; spleen pale, occasionally dark; lung dark, patchy and distended. No test substance-related changes were noted in animals sacrificed at the end of the observation period.

Table B.6.2-5: Acute oral toxicity in mice

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
Males				
10	0/0/5	-	-	0
71	0/5/5	10 m - 4 h	-	0
100	1/5/5	5 m - 3 h	55 m	20
120	2/5/5	5 m - 7 h	1 h	40
140	2/5/5	5 m - 7 h	10 m - 15 m	40
160	5/5/5	5 m - 55 m	10 m - 55 m	100
250	5/5/5	5 m - 1 h	20 m - 1 h	100
Females				
10	0/0/5	-	-	0
100	0/5/5	5 m - 6 h	-	0
120	1/5/5	5 m - 4 h	15 m	20
140	1/5/5	5 m - 7 h	15 m	20
160	2/5/5	5 m - 6 h	25 m - 35 m	40
250	5/5/5	5 m - 45 m	30 m - 45 m	100
LD_{50} mouse,	males: 131 mg/kg bw females: 168 mg/kg bw			

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Conclusion:
Following acute oral administration imidacloprid is more toxic in mice than in rats.

B.6.2.2 Percutaneous

Report: Kroetlinger, F. (1989)
NTN 33893 (c.n. imidacloprid (proposed)) - Study for acute dermal toxicity to rats.
Bayer AG, unpublished report No.: 18532, date: 15.11.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 402, FIFRA § 81-2, EEC B.3.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mixed batch 180587, purity: 94.2 % was used for testing. For each dose and animal, the solid test substance was weighed on an aluminium foil used to cover the administration site. The test substance was then mixed to a paste with 1.5 mL of sterile 0.9 % NaCl solution per g test substance and applied to the intact dorsal skin, shorn on the previous day, of 5 SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) per sex, respectively.

Findings:

Table B.6.2-6: Acute dermal toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death	Mortality [%]
Males						
5000	0	0	5	-	-	0
Females						
5000	0	0	5	-	-	0
LD_{50} rat, males and females: > 5000 mg/kg bw						

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Clinical signs: A dose of 5000 mg/kg bw of imidacloprid under 24-hour occlusive conditions was tolerated by Wistar rats of both sexes without clinical signs, body weight influences or mortalities.
Gross necropsy: No treatment-related findings.

Conclusion:
Imidacloprid is non-toxic to rats following dermal administration.

B.6.2.3 Inhalation

Report: Pauluhn, J. (1988a)
NTN 33893 - Study for acute inhalation toxicity in the rat in accordance with OECD Guideline No. 403.
Bayer AG, unpublished report No.: 16777, date: 6.6.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 403, FIFRA § 81-3, EEC B.2.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mixed batch 180587, purity: 95.3 % was used for testing. The test article was delivered in aerosol (nebulised with polyethylene glycol E 400 as vehicle) and dust form (undiluted) nose only to SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) for four hours.

Findings:

Table B.6.2-7: Acute inhalation toxicity in rats

Group	Concentration (nominal/analytical) (mg/m³ air)	Toxicological results#	Duration of signs	Time of death
Males				
1	Air control	0/0/10	-	-
2	vehicle control	0/0/5	-	-
3	500/69	0/0/5	-	-
4	--/1220	0/0/5	-	-
5	--/2577	0/5/5	4 h - 6 h	-
6	--/5323	0/5/5	4 h - 6 h	-
Females				
1	Air control	0/0/5	-	-
2	vehicle control	0/0/5	-	-
3	500/69	0/0/5	-	-
4	--/1220	0/0/5	-	-
5	--/2577	0/5/5	4 h - 6 h	-
6	--/5323	0/5/5	4 h - 6 h	-
LC_{50} rat males and females		(aerosol): > 69 mg/m³ air* (dust): > 5323 mg/m³ air*		

Group 1: 10 L air/minute;
Group 2: 20000 µL vehicle/m³ air (nominal) - 10 L air/minute;
Group 3: Nebulisation of a 2.5 % solution (g/v) - aerosol;
Group 4 - 6: Dust dispersion
1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group; * max. technically producible concentration

Clinical signs: Difficult breathing, reduced motility and piloerection (group 5 and 6); slight tremors (group 6).
Body weights: Marginal decrease of body weight gains in males (group 6) and in females (group 5); statistically significant decrease of body weight gains in females (group 6) during the post-treatment observation period.
Gross necropsy: No treatment-related findings

Conclusion:
Imidacloprid shows a low acute toxicity to rats following inhalation of aerosol or dust.

B.6.2.4 Skin irritation

Report: Pauluhn, J. (1988b)
NTN 33893 – Study for irritation/corrosive potential on the skin (rabbit) according to OECD Guideline No. 404.
Bayer AG, unpublished report No.: 16455, date: 25.2.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 404, FIFRA § 81-4, EEC B.4.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals:
Imidacloprid, batch no. 17001/87, purity: 94.2 % was used for testing. 500 mg of the undiluted test substance, mixed to a paste with water, was applied to the shorn skin of three rabbits (Strain HC:NZW; Breeder Interfauna, UK). The duration of exposure was 4 hours.

Findings:

Table B.6.2-8: Rabbit skin irritation study – Skin irritation grading

Animal no.	Draize grade after												Irrit.	
	1h		24h		48h		72h		7d		14d		Index	
	E	*O*	*E*	*O*	*E*	*O*	*E*	*O*	*E*	*O*	*E*	*O*	*E*	*O*
W1	1	0	0	0	0	0	0	0	0	0	-	-	0.0	0.0
W13	0	0	0	0	0	0	0	0	0	0	-	-	0.0	0.0
V25	0	0	0	0	0	0	0	0	0	0	-	-	0.0	0.0

- = not examined; d = day; E = erythema and eschar formation; h = hour; O = oedema formation

The results of the study show that the test substance does not possess a local irritant potential to the skin.

Conclusion:
Imidacloprid has no irritant effect to the skin.

B.6.2.5 Eye irritation

Report: Pauluhn, J. (1988c)
NTN 33893 – Study for irritant/corrosive potential on the eye (rabbit) according to OECD Guideline No. 405.
Bayer AG, unpublished report No.: 16456, date: 25.2.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 405, FIFRA § 81-5, EEC B.5.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, batch no. 17001/87, purity 94.2 % was used for testing. 100 µL (appr. 60 mg) test article was administered into the conjunctival sac of three rabbits (Strain HC:NZW; Breeder Interfauna, UK). The duration of exposure was 24 hours.

Findings:
The results of the study show that the test substance does not possess a local irritant potential to the eye.

Table B.6.2-9: Rabbit eye irritation study – Eye irritation grading and symptoms

Animal no.	Organ examined	Signs	Draize grades							Irrit. grade
			1h	24h	48h	72h	7d	14d	21d	
U56 f	Cornea	o	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	
	Iris		0	0	0	0	0	-	-	0.0
	Conjunctivae	R	2	0	0	0	0	-	-	0.0
		S	1	0	0	0	0	-	-	0.0
		T	0	0	0	0	0	-	-	
W54 m	Cornea	o	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	
	Iris		0	0	0	0	0	-	-	0.0
	Conjunctivae	R	1	0	0	0	0	-	-	0.0
		S	0	0	0	0	0	-	-	0.0
		T	0	0	0	0	0	-	-	
N31 m	Cornea	o	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	
	Iris		0	0	0	0	0	-	-	0.0
	Conjunctivae	R	1	0	0	0	0	-	-	0.0
		S	0	0	0	0	0	-	-	0.0
		T	0	0	0	0	0	-	-	

o: opacity; s = surface; - : not examined; m = male, f = female; R = redness, S = swelling, T = lacrimation

Conclusion:
Imidacloprid has no irritant effect to the eye.

B.6.2.6 Skin sensitisation

Report: Otha, K. (1988)
NTN 33893 technical – Study for skin sensitising effect on guinea pigs (Maximisation Test).
Bayer AG, unpublished report No.: 16533, date: 15.3.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 406, FIFRA § 81-6, EEC B.6.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, batch no. 17001/87, purity: 94.2 % was studied for skin sensitising potential in SPF-bred guinea pigs (Strain DHPW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). The test substance was formulated in sterile physiological saline solution containing 2 % Cremophor EL® to yield a suspension. A 1 % concentration was used for intradermal, and a 25 % concentration for topical induction. A 3 % and a 25 % concentration were used for challenge.

Findings:
Following the challenge neither the animals in the test article group, nor the animals in the control group exhibited any skin reactions.

Conclusion:
Imidacloprid has no skin sensitising potential under the conditions of the Maximisation test.

B.6.2.7 Acute intraperitoneal toxicity

Report: Kroetlinger, F. (1990)
NTN 33893 (c.n. *imidacloprid* [proposed] – Study for acute intra-peritoneal toxicity in rats.
Bayer AG, unpublished report no. 19245, date: 19.7.1990

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: Adapted for intraperitoneal administration

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid, mixed batch 180587, purity: 94.2 % was used for testing. The test article was formulated in sterile 0.9 % NaCl solution with the aid of Cremophor EL 2 % v/v and administered by i.p. injection to SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Application volume: 10 mL/kg bw.

Findings:
Clinical signs: Apathy, reduced motility, labored and accelerated breathing, spasms, periodic tremors, periodic twitching and narrowed eyelids, piloerection (only males), dyspnea and lacrimation. Individual animals also showed periodic spasm-like twitching, lying on side, closed eyelids or spastic gait.
Body weights: Temporary influences on body weights from a dose of 170 mg/kg bw (males) and from 180 mg/kg bw (females).
Gross necropsy: Findings in animals which died during the observation period included: lung with isolated patches; spleen pale; liver isolated dark, isolated white, rice grain to pin-head sized deposits on the liver; white or light beige rice grain to pin-head or larger sized deposits on the abdominal organs; isolated white, rice grain to pin-head sized deposits on the abdominal wall; animal no. 14: white, pin-head sized deposits on the intestinal tract; some clear or clear-reddish fluid in the abdominal cavitiy; animal no. 56 large amount of red fluid in abdominal cavitiy; small intestine reddened in isolated cases, in isolated cases contents slimy red to red-brown; animal no. 50: contents of small intestine yellow, slimy, and at some sites dark red. Animals sacrificed at the end of the post-treatment observation period showed no evidence of substance-related gross organ lesions.

Table B.6.2-10: Acute intraperitoneal toxicity in rats

Dose	Toxicological	Duration of signs	Time of death	Mortality

[mg/kg bw]	results*			[%]
Males				
10	0/0/5	-	-	0
100	0/5/5	5 m - 1 d	-	0
160	0/5/5	4 m - 2 d	-	0
170	4/5/5	3 m - 4 d	1 h 15 m - 2 h 30 m	80
180	4/5/5	2 m - 2 d	45 m - 1 h 30 m	80
200	4/5/5	4 m - 2 d	1 h 15 m - 2 h	80
250	5/5/5	4 m - 2 h 15 m	30 m - 2 h 15 m	100
500	5/5/5	4 m - 2 h 15 m	22 m - 2 h 15 m	100
Females				
10**	0/0/5	-	-	0
100	0/5/5	5 m - 1 d	-	0
150	1/5/5	4 m - 3 d	5 h	20
180	2/5/5	3 m - 3 d	3 h 30 m	40
200	2/5/5	3 m - 2 d	1 h 45 m - 3 h 30 m	40
224	5/5/5	2 m - 3 h 30 m	50 m - 3 h 30 m	100
500**	5/5/5	2 m - 3 h	1 h 30 m - 3 h	100
LD_{50} rat, males: 160 < LD_{50} < 170 mg/kg bw females: 186 mg/kg bw				

*1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Discussion:
The route of exposure is not a relevant one.

Conclusion:
Imidacloprid is highly toxic to rats following intraperitoneal administration.

B.6.3 Short-term toxicity (Annex IIA 5.3)

Reduced body weight gain was the most sensitive parameter in rats, mice and dogs following short term administration of imidacloprid. Transient trembling and severe tremor was observed in dogs from the first week of treatment in the 28-day and the 90-day studies but not in the one-year study which used similar dose levels as the 90-day study. The reasons for this dissimilarity are not entirely clear but are believed to be related to differences in exposure from food/ test substance consumption and induction of metabolic enzymes. The NOAEL for this endpoint from all available dog studies is considered to be 40 mg/kg bw.

In dogs and rats, the liver was the main target organ and an induction of microsomal enzymes was apparent from the elevated activites of the mixed-function oxidases, particularly that of cytochrome P-450. In conjunction with this, slight hepatocellular hypertrophy and cytoplasmic lesions were observed in histology. Elevated liver weights were occasionlly encountered. Higher doses led to an effect on hepatic function with dysregulation of the lipid and protein metabolism, as manifested by the changes of blood cholesterol, triglyceride, protein and albumin levels. The occasional increases in blood coagulation time and elevated total serum bilirubin levels are also attributed to disturbance of the hepatic function. Signs of a direct toxic effect on liver cells were observed only after administration of high doses.

These changes included elevated serum activities of ALT, AP and GLDH and histopathological findings such as swollen cellular nuclei, round-cell infiltration and hepatic cell necrosis.

Effects on the liver, including induction of mixed-function oxidases, elevated serum activities of ALT, AP and GLDH as well as dysregulation of the lipid and protein metabolism were also observed following inhalation exposure of rats to imidacloprid. No toxicity was observed in rabbits following repeated dermal administration of the limit dose of 1000 mg/kg bw/day.

Table B.6.3-1: Summary of short-term toxicity studies

Type of study	Animal species	Dose range tested	NOEL/NOAEL/ NOEC	Reference
90-day feeding	Rat	0-120-600-3000 ppm	m & f: 120 ppm (11.0/14.6 mg/kg bw/day)	Eiben, 1988
90-day feeding	Rat	0-150-600-2400 ppm	m/f: 150/600 ppm (14.0/83.3 mg/kg bw/day)	Eiben, 1989
15-week feeding	Mouse	0-120-600-3000 ppm	m/f: 600 ppm (~ 391/446 mg/kg bw/day)	Eiben, 1988
28-day feeding	Dog	0-200-1000-5000 ppm	m & f: 200 ppm (7.3 mg/kg bw/day)	Bloch et al., 1987
90-day feeding	Dog	0-200-600-1800/ 1200 ppm	m & f: 600 ppm (23.5 mg/kg bw/day)	Ruf, 1990
1-year feeding	Dog	0-200-500-1250/ 2500 ppm	m & f: 500 ppm (15 mg/kg bw/day)	Allen, 1989
5 x 6 h inhalation, (dust)	Rat	0-20-109-505 mg/m³ air	20 mg/m³ air (~ 5.4 mg/kg bw/day)	Pauluhn, 1988
4-week inhalation (dust)	Rat	0-5.5-30.5-191.2 mg/m³ air	5.5 mg/m³ air (2.4 mg/kg bw/day)	Pauluhn, 1989
15-day dermal	Rabbit	0-1000 mg/kg bw/day	1000 mg/kg bw/day	Flucke, 1990

B.6.3.1 Subacute oral toxicity

B.6.3.1.1 Dog

Report: Bloch, I., Frei, T., Luetkemeier, H., Vogel, W. and Wilson, J. (1987) 28-day oral range-finding toxicity (feeding) study with NTN 33893 tech. in the dog.
[redacted] unpublished report No.: R 4196, date: 9.10.1987

GLP: No

Guideline: In main accordance to OECD 409

Deviations: None

Acceptability: The study is considered to be acceptable as a range-finding study.

Material and Methods:
Test material and test animals:
Imidacloprid technical, purity 92.8 %, batch no. PT 2/86, was administered to groups of two male and two female pure-bred Beagle dogs (Breeder Laboratory Research Enterprises, Inc., Kalamazoo, USA) at concentrations of 0-200-1000 or 5000 ppm in pelleted diet for periods of up to 28 days. Body-weight related mean doses were: 0, 7.3, 31.0 and 49.0 mg/kg bw/day for males and females. Mean values do not represent very well the exposure situation in the high dose group. Due to a highly variable food intake in this group, imidacloprid doses on various days of the study ranged from 0-180 mg/kg bw.

Findings:
General observations: Appearance, behaviour and mortality were unaffected at levels up to and including 1000 ppm. Two animals died at the 5000 ppm dose, and the other two were sacrificed in moribund conditions. Symptoms appeared in the first week and consisted of ataxia, tremor and occasionally vomiting. In a single male dog tremors were observed on the first day of the study after an imidacloprid consumption of about 180 mg/kg bw; this animal was found dead on the next day. Surviving animals showed tremors on various days of the study whenever they had consumed a dose of imidacloprid of about 80 mg/kg bw or more on the previous day. Food consumption was unaffected at a dose of 200 ppm. Slight, transient reductions in food consumption were recorded at 1000 ppm and food intake as well as body weight was markedly reduced at 5000 ppm.
Haematology, sensory function, clinical chemistry, immunotoxicology, urinalysis: Hearing tests, ophthalmoscopic examinations, urinalyses and haematology indicated no treatment-related changes at doses up to and including 5000 ppm. Cytochrome P-450 values in the liver were slightly elevated in males and females at 1000 ppm. T3 was slightly decreased in one male and one female dog at 5000 ppm.
Gross pathology, organ weights, histopathology: Liver weight was increased in one female at 1000 ppm. Histopathologically, one male in the 1000 ppm group exhibited slight hepatocellular hypertrophy with slight pigmentation of the Kupffer stellate cells; one animal had minimal follicular atrophy of the thyroid. The alterations in clinical chemistry and histopathology observed at 5000 ppm group (hepatocellular atrophy, thymus involution) are possibly effects associated with the severe disturbances in general conditions.

Conclusion:
NOAEL: 200 ppm (equivalent to 7.3 mg/kg bw/day) based on liver and thyroid effects (hepatocellular hypertrophy, follicular atrophy of thyroid) at 1000 ppm.

B.6.3.2 Subchronic oral toxicity

B.6.3.2.1 Rat

Report: Eiben, R. (1988)
NTN 33893 – Pilot range-finding study for a chronic toxicity study on Wistar rats (ninety-eight day feeding study).
Bayer AG, unpublished report No.: 17279, date: 24.10.1988

GLP: No

Guideline: In main accordance to: OECD 408, 67/548/EEC, US-EPA-FIFRA § 82-1

Deviations: No ophthalmoscopy

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals: Imidacloprid (purity: 92.8 %; batch no. 2/86 part 1) was administered to groups of 10 male and 10 female SPF-Cpb Wistar rats (Strain Bor: WISW, Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) in their diet at concentrations of 0, 120, 600 and 3000 ppm for up to 98 days. Mean consumption of imidacloprid per kg body weight and day were: 11, 56.9 and 408.9 mg for males and 14.6, 77.8 and 513.2 mg for females.

Findings:
General observations: No increase of mortality and no treatment-related effect on the general condition, appearance or behaviour were observed at doses up to and including 3000 ppm. Food intake was increased at 3000 ppm with minimal increases seen already at 600 ppm. The results of the water intake determinations did not vary significantly at doses up to 3000 ppm. The body weight development was minimally decreased at 600 ppm (males: -6 % to -7 %; females: -10 %) and significantly depressed in the 3000 ppm dose groups (males: -20 %; females: -15 %) in comparison to the control group animals.

Figure B.6.3-1: Pilot feeding study on rats - Body weights (mean values) - males

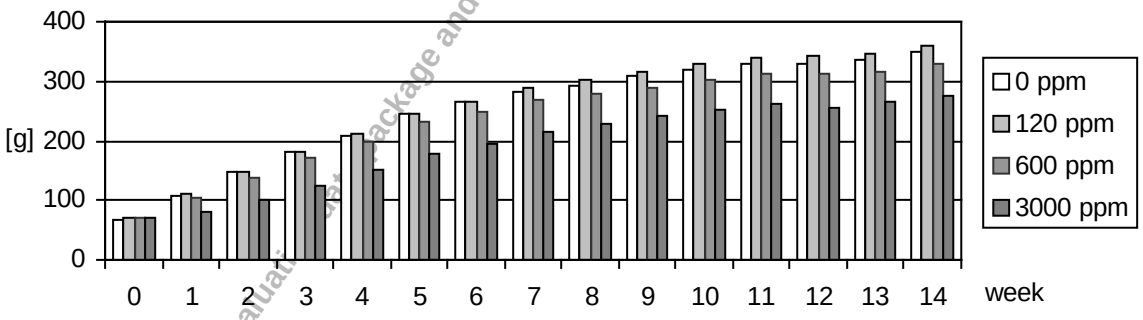
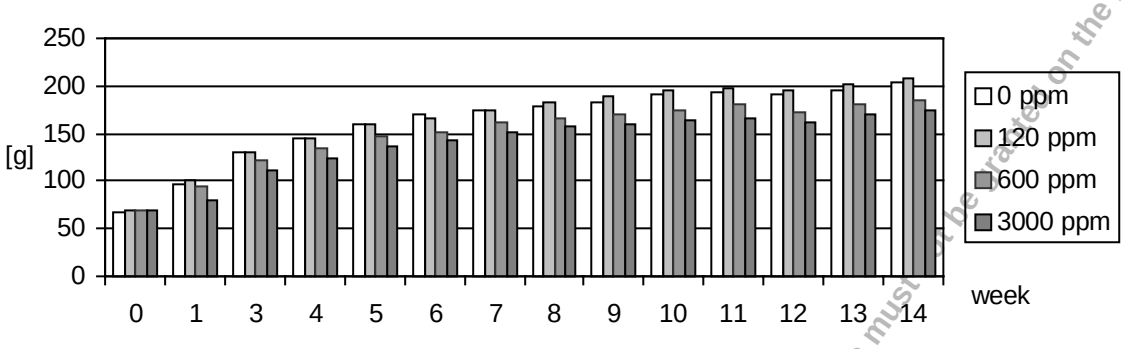


Figure B.6.3-2: Pilot feeding study in rats - Body weights (mean values) - females



Haematology, clinical chemistry, urinalysis: No damage to blood or haematopoietic organs may be inferred from the results of the haematological tests. Significantly elevated alkaline phosphatase and depressed glucose levels were determined in males and females of the 3000 ppm dose group and males showed also lower cholesterol levels. No treatment-related effects in urinalysis were observed.

Table B.6.3-2: Pilot feeding study in rats - Haematology and clinical chemistry

Week 12	**0 ppm**		**120 ppm**		**600 ppm**		**3000 ppm**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
AP [U/L]	259	202	290	205	295	215	348++	237++
Glucose [mmol/L]	5.94	5.81	5.62	5.78	5.66	5.65	5.26++	5.64+
Chol [mmol/L]	2.46	1.83	2.42	2.02	2.43	2.28	1.78++	2.07+

+ $p \leq 0.05$; ++ $p \leq 0.01$ (Mann-Whitney U-Test, two-tailed)

Gross pathology, organ weights, histopathology: The gross pathological observations gave no indications of treatment-related organ changes. No weight differences which would indicate organ injury are apparent at doses up to 3000 ppm. Histopathologically, degenerative changes in the epithelium of the testicular tubuli were apparent in 5 out of 10 males of the 3000 ppm dose group; spermatogenesis was not negatively affected. A multifocal cell necrosis was diagnosed in the liver of one male in the 3000 ppm dose group. Also there is evidence that isolated liver cell necrosis could be increased in the high dose group. There were no indications of toxic effects on the other organs examined.

Conclusion:
NOAEL: 120 ppm (equivalent to 11 mg/kg bw/day for females and 14.6 mg/kg bw/day for males) based on slightly increased food consumption and inhibited body weight development at 600 ppm.

Report: Eiben, R. (1989)
NTN 33893 - Subchronic toxicity study on Wistar rats
(Administration in the feed for 96 days).
Bayer AG, unpublished report No.: 18187, date: 14.7.1989

GLP: Yes (certified laboratory). Deviation: none

Guideline: OECD 408, 67/548/EEC, US-EPA-FIFRA § 82-1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and Test animals: Imidacloprid technical, batch no 180587, purity: 95.3 %, was administered to groups of 10 male and 10 female Wistar rats (Strain Bor: WISW, Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) at concentrations of 0, 150, 600 or 2400 ppm in the diet for periods of up to 96 days. Mean consumption of imidacloprid per kg body weight and day was 14.0, 60.9 or 300.2 mg for males and 20.3, 83.3 or 422.2 mg for females. Additional groups consisting of 10 male and 10 female rats received the test substance at concentrations of 0 or 2400 ppm in the diet over 96 days to study the reversibility of effects within a four-week period during which control food was provided.

Findings:
General observations: Appearance, behaviour, water intake and viability were unaffected at doses up to and including 2400 ppm. The food consumption was slightly increased (7-12 %) at levels up to and including 600 ppm. At a dose of 2400 ppm feed intake was increased during the treatment and recovery periods. Reduced body weight gains were observed in males at 600 ppm, and in both sexes at 2400 ppm. A slight reduction in the body weight differences between the 0 ppm and 2400 ppm group animals was observed in the recovery groups during the four-week post-treatment observation period. No treatment-related changes in the eyes were found at ophthalmic and histological examinations in males and females at doses up to and including 2400 ppm.

Figure B.6.3-3: Subchronic feeding study in rats
Body weights (mean values) - males

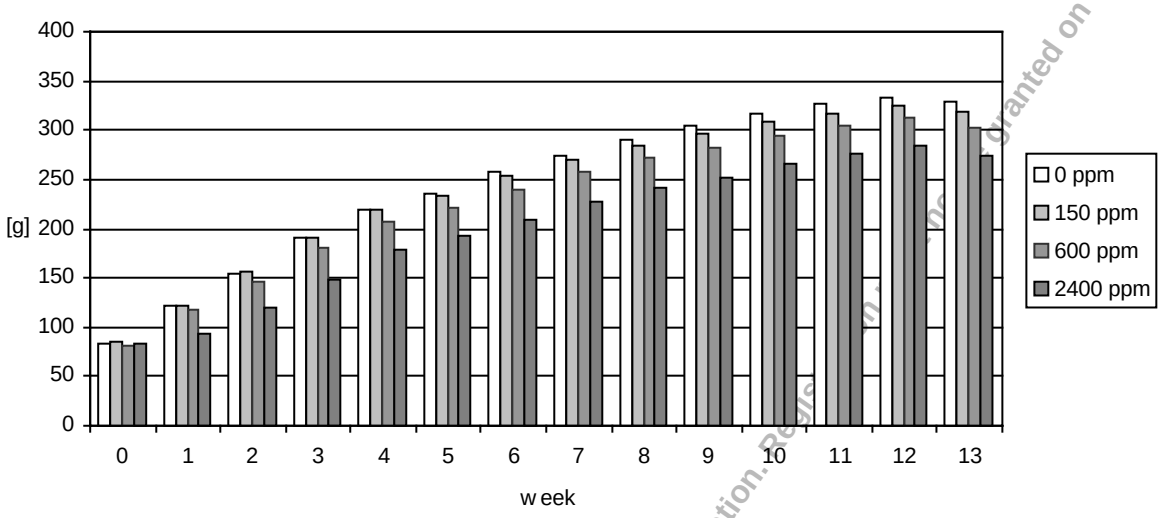
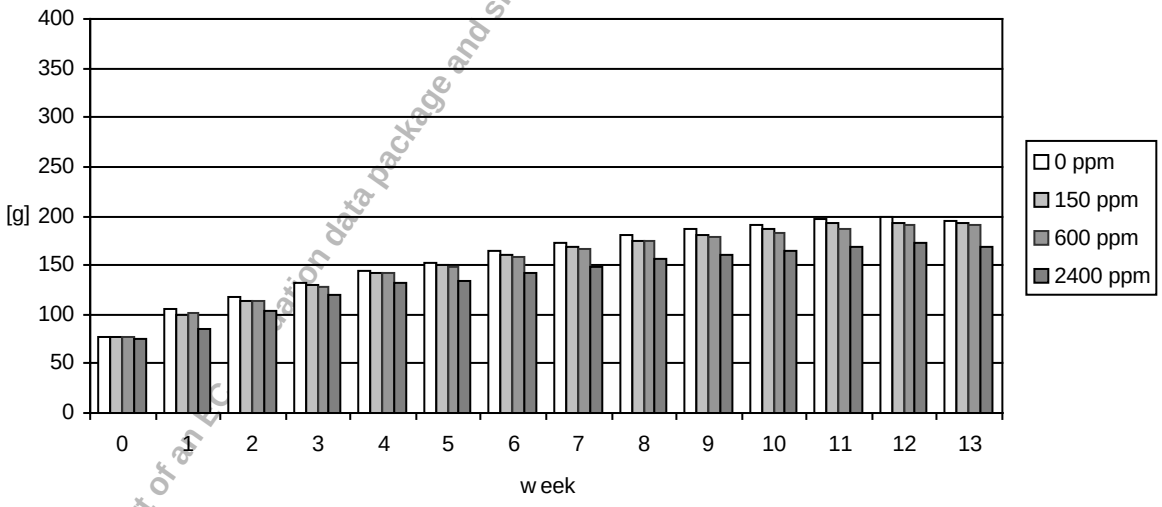


Figure B.6.3-4: Subchronic feeding study in rats
Body weights (mean values) - females



Haematology, clinical chemistry, urinalysis: At doses up to and including 2400 ppm there were no signs of treatment-induced effects on the haematogenic organs. Slightly longer thromboplastin times and depressed thrombocyte counts were found at 2400 ppm. Both findings were only partially reversible within the post-treatment observation period. The plasma, erythrocyte and brain cholinesterase activities were not affected. Urinalysis and histopathological data provided no evidence of renal impairment. Slightly elevated AP and ALAT activities as well a depressed protein, albumin, cholesterol and triglyceride levels were

determined in the males at 2400 ppm and were considered indicative of liver toxicity. The slightly depressed protein, albumin and cholesterol levels determined in the females at 2400 ppm were assessed as an expression of functional liver impairment.

Table B.6.3-3: Subchronic feeding study in rats - Haematology and clinical chemistry

Week 14	**0 ppm**		**150 ppm**		**600 ppm**		**2400 ppm**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Thro [giga/L]	956	910	932	874	918	917	903	843
Hquick [sec]	29.6	27.7	30.2	26.6	31.4	28.9	32.6++	29.5++
AP [U/L]	277	176	268	172	248+	185	292	190
ALAT [U/L]	42.4	35.6	41.5	37.5	42.9	35.4	52.8++	39.6
Prot [g/L]	67.2	62.8	64.8++	62.8	64.0+	62.2	62.1++	59.4+
Albumin [g/L]	31.5	33.3	31.2	33.2	30.6	32.3	30.6+	31.2+
Chol [µmol/L]	2.31	1.71	1.97	1.75	2.08	1.78	1.88+	1.65
Trigl [mmol/L]	1.56	0.90	1.25	1.17+	1.29	0.76	0.75++	0.94

+ = $p \leq 0.05$; ++ = ≤ 0.01 (Mann-Whitney U-Test, two-tailed)

Gross pathology, organ weights, histopathology: Histopathological findings in the livers of males at 2400 ppm were elevated incidence of cellular necroses, round-cell infiltration, swollen cellular nuclei, cytoplasmic lesions. This indicates a hepatoxic effect which was shown to be reversible within the post-treatment observation period. No evidence for adverse effects in other organs was seen at doses up to and including 2400 ppm.

Conclusion:
NOAEL: 150/600 ppm, equivalent to 14.0 mg/kg bw/day for males and 83.3 mg/kg bw/day for females, based on decreased body weight gain at 600 ppm (males) and 2400 ppm (females) and functional changes in the liver at 2400 ppm in females.

B.6.3.2.2 Mouse

Report: Eiben, R. (1988)
NTN 33893 – Pilot range-finding study for a cancerogenesis study on B6C3F1 mice (one hundred seven day feeding study).
Bayer AG, unpublished report No.: 17280, date: 24.10.1988

GLP: No

Guideline: In main accordance to: OECD 408, 67/548/EEC, US-EPA-FIFRA § 82-1

Acceptability: The study is considered to be acceptable as a range finding study.

Material and Methods:
Test material and test animals:
Imidacloprid (purity: 92.8 %; batch no. 2/86 part 1) was administered to groups of 10 male and 10 female Charles-River B6C3F1 mice (Breeder Charles River Wiga GmbH, Sulzfeld, Germany) in their diet at concentrations of 0, 120, 600 and 3000 ppm for up to 107 days. Mean consumption of imidacloprid per kg body weight and day was 77.4, 391.2 or 2408.0 mg for males and 89.4, 445.7 or 3087.2 mg for females.

Findings:

General observations: Imidacloprid concentrations up to 600 ppm had no effect on the viability. Seven males and seven females died at 3000 ppm related to blood withdrawal during week 13. Several animals displayed poor general conditions, and rough coats were frequently observed at 3000 ppm. At 600 ppm (males) and at 3000 ppm (males and females) the body weight gain was reduced and the food consumption was increased. However, males in the 600 ppm group had lower starting weights than the other groups and any findings on body weights and food consumption may be related to this difference rather than to exposure to imidacloprid.

Figure B.6.3-5: Pilot feeding study on mice: Body weights (mean values) - males

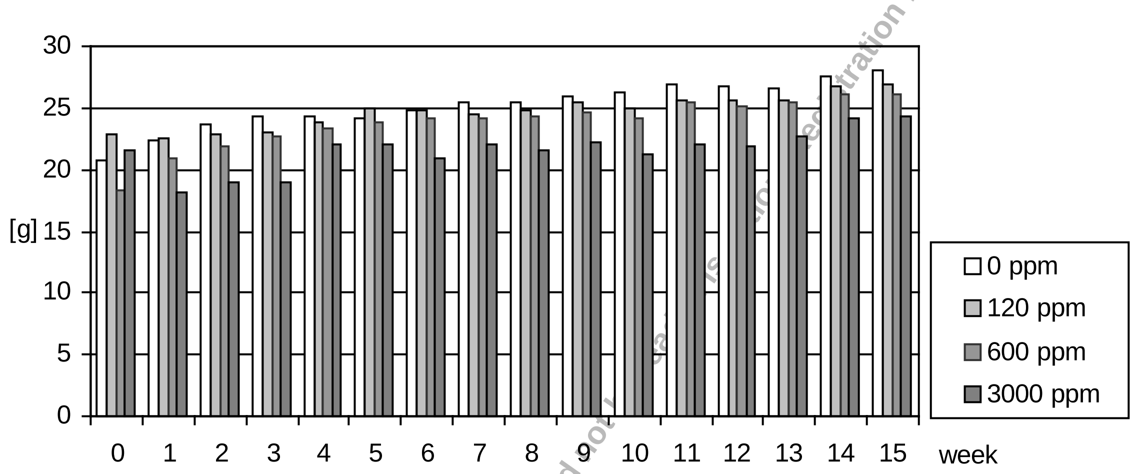
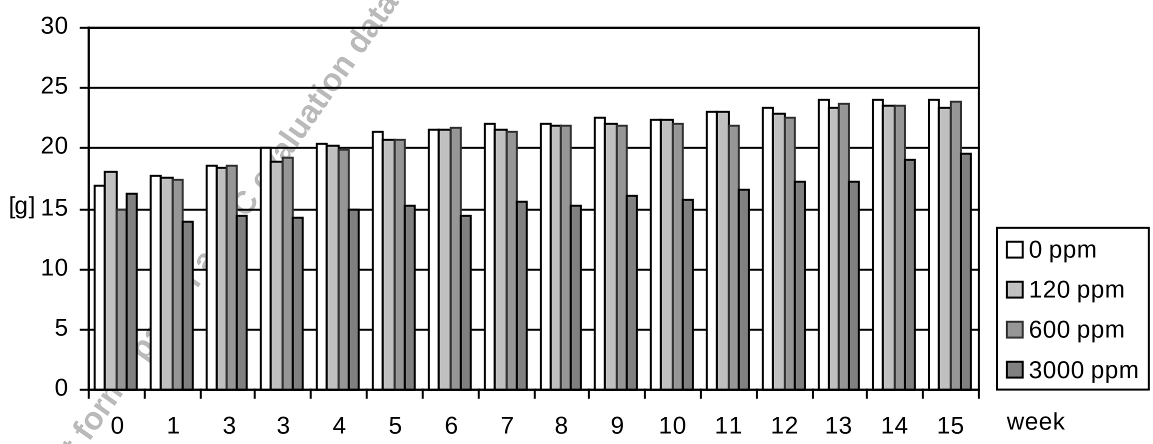


Figure B.6.3-6: Pilot feeding study in mice: Body weights (mean values) - females



Haematology, clinical chemistry, urinalysis: No signs of treatment-related haematological changes were observed at 3000 ppm. At 3000 ppm clinical chemistry laboratory tests showed significantly decreased urea and cholesterol levels (males), as well as lower alanine aminotransferase and glucose values (females). The alkaline phosphatase activities were significantly increased in males and females.
Gross pathology, organ weights, histopathology: Organ weight differences observed at 3000 ppm (liver, heart, spleen, kidneys, testes, adrenals) were attributed to the distinct body weight decreases. There were no treatment-related histopathological findings.

Table B.6.3-4 Pilot feeding study in mice - Clinical chemistry

Week 12	**0 ppm**		**120 ppm**		**600 ppm**		**3000 ppm**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
ALAT [U/L]	36.2	49.3	51.0++	53.5	43.3	41.0	32.5	30.0++
AP [U/L]	106	161	105	176+	114	184++	151++	237++
Cholesterol [mmol/L]	2.69	2.17	2.36++	2.09	2.36++	2.18	2.10++	1.83
Glucose [mmol/L]	6.35	5.68	6.61	6.11	6.37	5.64	6.01	4.51+
Urea [mmol/L]	11.07	10.25	10.85	9.81	10.08	8.82+	7.52++	8.72

+ = $p \leq 0.05$; ++ = ≤ 0.01 (Mann-Whitney U-Test, two-tailed)

Conclusion:
NOAEL: 600 ppm (~ 391 mg/kg bw/day for males, ~ 446 mg/kg bw/day for females) based on body weight loss and decreased body weight gain at 3000 ppm.

B.6.3.2.3 Dog

Report: Ruf, J. (1990)
NTN 33893 technical - Subchronic Toxicity Study on dogs in oral administration (Thirteen-week feeding study).
Bayer AG, unpublished report No.: 18732, date: 2.2.1990

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 409

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material and test animals:
Groups of four male and four female beagle dogs (Strain Bor:Beag, Breeder F. Winkelmann, Borchen, Germany) were administered imidacloprid, batch no. 180587, purity 95.3 % at concentrations of 0, 200, 600 or 1800/1200 ppm in their diet (mash, prepared from powdered feed with equal amount of water) for a period of 13 weeks. The active ingredient level in the highest dose group was reduced from week four because of low food intakes. Mean consumption of imidacloprid per kg body weight and day was 7.8, 23.5 and 45.4 mg in the three dose groups. Food consumption and test substance intake data are not available on a

daily basis but are reported weekly which does not give an appropriate representation of exposure conditions for the high dose group.

Findings:
General observations: Appearance, behaviour and food consumption were unaffected at 200 ppm and no mortality was observed at any dose level. Body weight development was unaffected at 600 ppm, although food consumption was reduced in two animals at this dose level. Body weight loss occurred at 1800 ppm. After the active ingredient concentration had been reduced to 1200 ppm, a trend towards normalisation became apparent but body weight gain remained reduced in the high dose group. The effect on food consumption was probably due to a palatability problem; the animals in the high dose group group (after reduction to 1200 ppm) completely consumed the food provided to them after a more tasty dog food had been blended into it on a test basis.
At doses of 600 ppm and higher, some animals also exhibited a clinically emaciated state of nutrition (although this was not confirmed during necropsy at the end of the study) and all dogs showed transient trembling (600 ppm) or severe tremors (at 1800/1200 ppm) during the first month of the study. Tremors were noted already during the first week of treatment and are considered to be acute effects based on the findings in the 28-day study. It can be assumed that, as in the 28-day study, food consumption was highly variable in the high dose group during the first four weeks and that the tremors developed when a dog consumed nearly the maximum amount of test substance available (80 mg/kg bw) on a given day. After the dose had been reduced to a maximum of about 53 mg/kg bw/day slight trembling was still observed in a single high dose dog but tremors did not occur in the second part of the study. These observations correlate well with the findings in the 28-day study. The presentation of clinical signs, food consumption and substance intake by week and not by day of observation in the study report of this 90-day study, however, precludes a day-by-day comparison of food intake patterns/test substance consumption with signs of neurotoxicity.

Figure B.6.3-7: 13-week feeding study in dogs - Body weights (mean values) - males

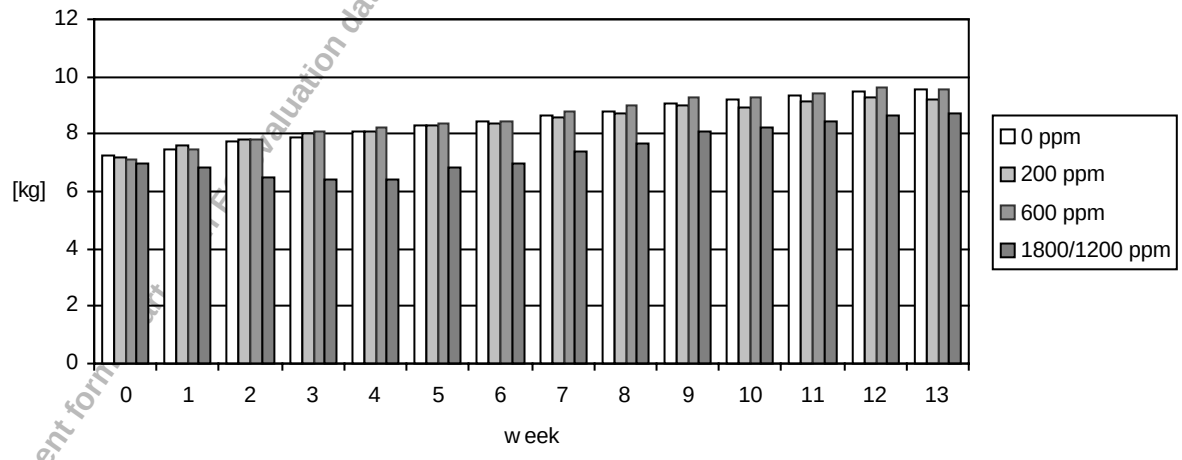
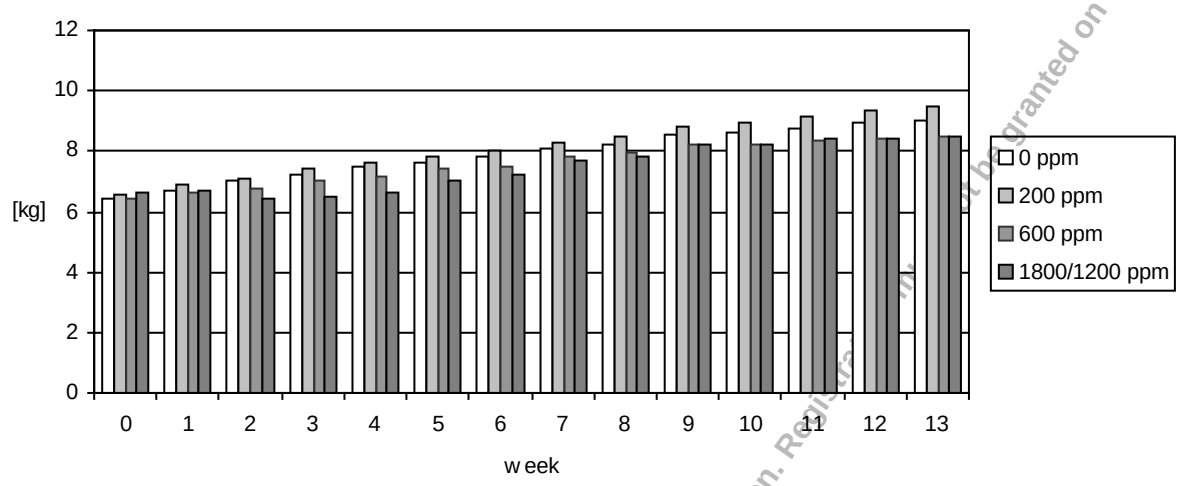


Figure B.6.3-8: 13-week feeding study in dogs: Body weights (mean values) - females



Haematology, clinical chemistry, urinalysis: There were no indications of haematotoxicity or damage to the haematogenic organs. Also clinical chemistry, organ gravimetry and urinalysis gave no evidence for adverse effects.
Gross pathology, organ weights, histopathology: Neither the necropsy nor the histopathological examinations resulted in any findings attributable to the test substance treatment.

Conclusion:
Effects on food consumption and body weight development are regarded mainly as a palatability problem and not as toxic effects. The only other relevant finding was the occurrence of tremors in the high dose group early during the study. The more subtle effects at 600 ppm are not considered to be truely adverse due to their sporadic occurrence during the first week of the study only. The shortcomings of the reporting do not allow to identify the actual doses that induced tremors in individual animals of the high dose group which are probably higher than the values derived from averaging food and substance intakes over a week. However, as these data are lacking, a NOAEL of 600 ppm (23.5 mg/kg bw/day) is proposed based on clinical signs (tremor) at 1800/1200 ppm, equivalent to 45.4 mg/kg bw/day.

Report:	Allen, T.R., Frei, Th., Luetkemeier, H., Vogel, O., Biedermann, K. and Wilson, J. (1989) 52-week oral toxicity (feeding) study with NTN 33893 technical in the dog. [redacted] unpublished report No.: R 4856, date: 19.10.1989 *+ Amendment report No.: R 4856 A, date: 3.3.1992.*
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	OECD 452; FIFRA § 83-1;
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity 94.9 %
Test animals: Beagle dog (Breeder Laboratory Research Enterprises, Inc., Kalamazoo, MI 49009, U.S.A.)
Groups of 4 male and 4 female pure-bred beagle dogs were administered imidacloprid at concentrations of 0, 200, 500 or 1250/2500 ppm in their diet for a period of 52 weeks. The concentration of 1250 ppm was increased to 2500 ppm from treatment week 17 onwards. Mean uptakes of imidacloprid were 0, 6.1, 15 or 41/72 mg/kg bw/day for males and females combined.

Findings:
General observations: Appearance, behaviour, body weight gains and mortality were unaffected in males and females at 1250/2500 ppm. Initial slight reductions in food intake were observed in both sexes at 1250 ppm and when the concentration was increased to 2500 ppm. Ophthalmic examinations and hearing tests indicated no treatment-related changes at 1250/2500 ppm doses. In contrast to the findings of the 90-day study no indications of trembling or tremor were observed which supports a NOAEL for this endpoint of 40-50 mg/kg bw/day. Due to the fact that enzyme induction was observed in the high dose group the increase of the test substance concentration in the food did not necessarily result in much higher plasma levels of the active substance after week 17.

Haematology, clinical chemistry, urinalysis: There were no treatment-related effects on haematology test parameters up to a dose of 1250/2500 ppm. At 1250/2500 ppm a slight increase in the plasma cholesterol levels in the females and slight increases in the hepatic cytochrome P-450 values in males and females were observed. No treatment-related effects were seen on urinary parameters.

Table B.6.3-5: 1-year study on dogs – Clinical chemistry and liver weights

Dose	**0 ppm**			**200 ppm**			**500 ppm**			**1250/2500 ppm**		
Week	**13**	**26**	**52**	**13**	**26**	**52**	**13**	**26**	**52**	**13**	**26**	**52**
Males												
Absolute liver weights [g]			317.0			302.1			297.0			339.5
Cyt. P-450 [nmol/g]	--	--	13.2	--	--	18.2	--	--	16.0	--	--	25.6 ++
Cholesterol [mmol/L]	3.45	3.69	4.13	4.24	4.07	3.94	4.37	4.35	4.23	4.56	4.53	4.04
Females												
Absolute liver weights [g]			268.5			302.4			268.0			320.0
Cyt. P-450 [nmol/g]	--	--	14.6	--	--	15.0	--	--	18.0	--	--	22.0 +
Cholesterol [mmol/L]	3.39	3.75	4.08	3.43	3.91	3.69	4.17	4.93	4.23	4.90 ++	6.07 +	6.82

+ = $p \leq 0.05$; ++ = $p \leq 0.01$ (Dunnett-test based on pooled variance)

Gross pathology, organ weights, histopathology: Slightly elevated liver weights which were observed at 1250/2500 ppm can be seen as an adaptation process of the organ for the metabolisation of imidacloprid. The gross pathology and histopathology produced no evidence for treatment-related changes in the organs and tissues examined. No further organ weight changes occurred.

Conclusion:
NOAEL: 500 ppm, equivalent to 15 mg/kg bw/day based on liver effects (slight increase of weight, plasma cholesterol and cytochrome P-450) at 1250/2500 ppm.

B.6.3.3 Other routes

B.6.3.3.1 Subacute inhalation toxicity

Report: Pauluhn, J. (1988a)
NTN 33893 – Study for acute inhalation toxicity in the rat in accordance with OECD Guideline No. 403.
Bayer AG, unpublished report No.: 16777, date: 6.6.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: Special study

Acceptability: The study is considered to be acceptable for dose-range finding.

Material and Methods:
Test material and test animals:
Imidacloprid, mixed batch 180587, purity: 95.3 %. The test article was delivered in dust form (undiluted) nose only to groups of 10 male and 10 female Wistar rats (Strain Bor: WISW, Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) at analytically determined concentrations of 0, 20, 109 and 505 mg/m³ air on 5 consecutive days for 6 hours per day. The respirability of the test substance dust was relatively low at the highest concentration; only about 20 % of the administered mass of substance was in the form of particles less than 5 µm in size.

Findings:
General observations: All rats tolerated the treatment without symptoms. No mortalities occurred. A slight, transient effect on the body weight development were observed at exposure from 109 mg/m³ air onwards.

Haematology, clinical chemistry, urinalysis: From 109 mg/m³ air induction of mixed-function oxidases occurred. There were no indications of a hepatic effect in ALAT, ASAT or GLDH.

Table B.6.3-6: Subacute inhalation study in rats - Clinical chemistry

Dose Group	**Air control**		**20 mg/m³ air**		**109 mg/m³ air**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*
O-DEM [mU/g]	156.1	58.1	160.9	67.7	200.6	140.7
N-DEM [mU/]	9.9	8.0	9.4	7.8	11.6	10.8

Organ weights: No relevant effects.
Gross pathology, organ weights, histopathology: No histopathological evidence for specific adverse effects were found in any of the organs examined.

Conclusion:
NOAEC: 20 mg/m³ air, based on transient decrease in body weight gain at 109 mg/m³ air.

Report: Pauluhn, J. (1989)
NTN 33893 (proposed common name: *imidacloprid*) – Subacute inhalation toxicity study on the rat according to OECD Guideline No. 412.
Bayer AG, unpublished report No.: 18199, date: 18.7.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 412, FIFRA § 82-4, 87/302/EEC.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

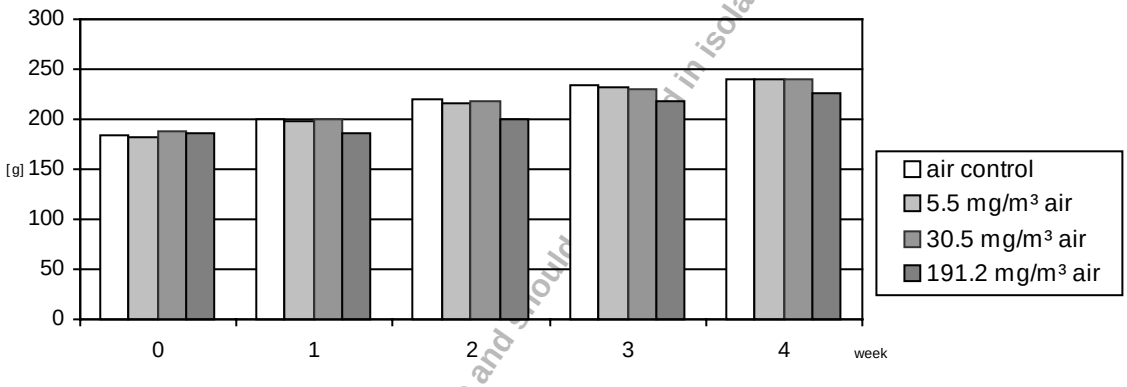
Test material and test animals:

Groups of 10 male and 10 female Wistar rats (Strain Bor: WISW, Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) were exposed nose-only to imidacloprid, mixed batch 180587, purity: 95.2 %, at mean analytical dust concentrations of 5.5, 30.5 and 191.2 mg/m³ air for a period of 4 weeks (6 hours daily, 5 days per week). The mass-related potential "respirability" (particle size ≤ 5 µm) in the dose groups was 95, 53 and 43 %, respectively.

Findings:

General observations: All rats tolerated the treatment without test substance-induced symptoms or mortality. The body weight gains were unaffected in females at exposures up to and including 191.2 mg/m³ air, and in males at doses up to and including 30.5 mg/m³ air. The body weight gains of the males were reduced at the 191.2 mg/m³ air level.

Figure B.6.3-9: Subacute inhalation study in rats - Body weights (mean values) - males



Haematology, clinical chemistry, neurology: Elevated mixed-function oxidase activities were found in the liver homogenate of females at 30.5 mg/m³ air and above, and in males at 191.2 mg/m³ air. The depressed serum triglyceride levels found in both sexes at 191.2 mg/m³ air as well as most of the other findings are considered to be related to effects on liver function. Elevated ALAT values, elevated alkaline phosphatase (AP) activities and depressed plasma CHE levels were observed in females at from 30.5 mg/m³ air; increased GLDH activities were seen in both sexes at 191.2 mg/m³ air. The serum α1-globulin fraction was reduced in both sexes at concentrations from 30.5 mg/m³ air onwards. The urinary pH was elevated in the females at 191.2 mg/m³ air. The females exhibited an increase in the blood coagulation time as well as an elevated total serum bilirubin level at 191.2 mg/m³ air. These changes are considered related to the effects on the liver. The thrombocyte counts were depressed in both sexes at 191.2 mg/m³ air.

Table B.6.3-7: Subacute inhalation study in rats - Haematology and clinical chemistry, relative organ weights

Week 4	**Air control**		**5.5 mg/m³ air**		**30.5 mg/m³ air**		**191.2 mg/m³ air**	
	Males	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	*Females*
Trigl [mcmol/g]	6.20	6.19	6.15	6.09	6.78	5.89	6.34	5.76
O-DEM [mU/g]	10.1	9.5	9.4	8.3	9.9	9.4	18.5++	11.5+
N-DEM [mU/]	130.4	59.7	115.5	59.7	120.2	75.6+	197.2++	105.4++
P450 [nmol/g]	41.7	37.2	44.9	42.2	44.9	36.7	55.8++	39.1
Serum trigl [mmol/L]	0.80	0.96	0.77	0.56	0.66	0.64	0.41+	0.26++
ALAT [U/L]	40.1	44.5	40.1	42.1	37.0	55.5++	39.8	75.8++
GLDH [U/L]	1.5	1.9	1.1	1.8	2.2	3.8	5.0+	13.9++
AP [U/L]	370	191	404	208	394	230+	410	279++
Thro [10E9/L]	970	929	906	953	939	955	824++	886
HQUICK [sec]	34.2	32.6	34.4	32.5	35.2	32.9	35.5	35.9++
CHE [kU/L]	0.43	1.42	0.42	1.52	0.43	1.05	0.44	1.02
α1-globlin [%]	18.9	17.0	19.0	17.0	17.5++	15.8+	16.7++	15.6+
Relative organ weights [mg/100 g bw]								
liver	3991	3541	3873	3542	3709	3853	3871	3980+
heart	350	356	325++	355	326++	345	341	337++
thymus	122	123	126	101	113	99	101	93+

+ = $p \leq 0.05$; ++ = $p \leq 0.01$ (Mann-Whitney U-Test, two-tailed)

Gross pathology, organ weights, histopathology: Only in females the relative liver weights were increased at 30.5 mg/m³ air and above. Slightly reduced relative heart and thymus weights were determined in females at 191.2 mg/m³ air. No histopathological evidence for specific adverse effects were found in any of the organs examined.

Conclusion:
NOAEC: 5.5 mg/m³ air, equivalent to 2.4 mg/kg bw/day, based on effects on the liver (slight weight increase and enzyme induction) at 30.5 mg/m³ air.

B.6.3.3.2 Subacute dermal toxicity

Report: Flucke, W. (1990)
NTN 33893 techn. – Study for subacute dermal toxicity in the rabbit.
Bayer AG, unpublished report No.: 19152, date: 11.6.1990

GLP: Yes (certified laboratory); Deviations: none

Guideline: OECD 410, FIFRA § 82-2.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:

Test material and test animals:

Groups of 5 male and 5 female New Zealand rabbits of the strain HC:NZW (Breeder Interfauna, UK) were administered imidacloprid, mixed batch 180587, purity: 95.0 %, at levels of 0 and 1000 mg/kg bw by dermal application. The test article was mixed to a paste using physiological saline solution containing 2 % Cremophor EL® and was applied to the shorn dorsal and flank skin of the rabbits for 6 hours per day over a period of 15 days (3 weeks, 5 days/week).

Findings:

Appearance, behaviour, feed consumption and body weights of the treated animals corresponded to that of the control animals. There were no test substance-induced mortalities. No treatment-related local skin findings were observed. The skin fold thickness of the treated animals did not differ from that of controls. No treatment-related haematological or clinical chemistry effects occurred. No treatment-related changes to the examined organs and tissues were observed in terms of gross pathological, gravimetric or histopathological findings.

Conclusion:

NOEL: 1000 mg/kg bw/day (systemic and local), limit dose tested.

B.6.4 Genotoxicity (Annex IIA 5.4)

Imidacloprid was tested for point-mutagenic activity, for chromosome aberration in vitro and in vivo and for DNA repair. All in vitro tests for point-mutation effects and the UDS test were negative. Very weak indications of sister chromatid exchange induction were found in one SCE/CHO test system in vitro at cytotoxic concentrations. This could not be confirmed in a second test in another laboratory, however, the concentrations tested were lower than in the first experiment. In addition, no increase of SCE in bone marrow was seen in the in vivo test. In a cytogenetic study on human lymphocyte cultures, a slight, reproducible increase in the aberration rate was observed in the cytotoxic concentration range without metabolic activation only; an equivocal result was obtained with metabolic activation. In vivo cytogenetic tests in mice and hamsters did not reveal a genotoxic potential of imidacloprid. Likewise, imidacloprid proved negative in a mouse bone marrow micronucleus assay. Since an autoradiography in rats gave evidence for the exposure of bone marrow it can be concluded that imidacloprid exhibits no mutagenic potential in vivo.

Table B.6.4-1: Summary of genotoxicity tests with imidacloprid

Test system in vitro	**Max. concentration/ dose**	**Purity (%)**	**Results**	**Reference**
Salmonella/microsome test	12500 µ/plate	95.0 %	negative	Herbold, 1989a
Salmonella/microsome test	5000 µg/plate	96.0 % 96.3 %	negative	Herbold, 1991
Salmonella/microsome test	5000 µg/plate	97.4 %	negative	Herbold, 1992
Salmonella, E. coli/microsome test	5000 µg/plate	93.7 %	negative	Watanabe, 1991a
B. subtilis recombination assay	5000 µg/plate	94.7 %	negative	Watanabe, 1990a
CHO-HGPRT	125 µg/mL (with S9-mix) 1222 µg/mL (without S9-mix)	95.2 %	negative	Lehn, 1989a
S. cerevisiae mitotic recombination	10000 µg/mL	95.3 %	negative	Herbold, 1988a
Rat hepatocyte unscheduled DNA synthesis (UDS)	750 µg/mL	95.2 %	negative	Cifone, 1988
CHO sister chromatid exchange	5000 µg/mL	95.2 %	**positive**	Taalman, 1988
CHO sister chromatid exchange	1250 µg/mL (with S9-mix) 400µg/mL (without S9-mix)	95.2 %	negative	Putmann & Morris, 1989
Human lymphocyte cytogenetic study	5200 µg/mL	95.2 %	**positive**	Herbold, 1989b
Test system in vivo				
Hamster cytogenetic study	2000 mg/kg bw	94.6 %	negative	Herbold, 1989c
Mouse micronucleus test	80 mg/kg bw	95.3 %	negative	Herbold, 1988b
Hamster sister chromatid exchange	2000 mg/kg bw	95.0 %	negative	Herbold, 1989d
Mouse cytogenetic study	80 mg/kg bw	94.1 %	negative	Völker, 1990

B.6.4.1 *In vitro* genotoxicity

Report: Herbold, B. (1989a)
NTN 33893 - Salmonella/microsome test to evaluate for point mutagenic effects.
Bayer AG, unpublished report No.: 17577, date: 6.1.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.0 %
Imidacloprid was tested in the Salmonella/microsome assay at concentrations of up to and including 12500 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.

Findings:
Imidacloprid concentrations up to 6200 µg/plate did not produce an increase in the mutant count. The total bacteria counts remained unchanged. No inhibition of growth was observed. At higher doses, the substance had a very weak strain-specific bacteriotoxic effect; this range could not be used for evaluation purposes.

Conclusion:
Imidacloprid is considered to be non-mutagenic in this assay with and without metabolic activation.

Report: Herbold, B. (1991)
NTN 33893 AMP – Salmonella/microsome test.
Bayer AG, unpublished report No.: 20090, date: 22.3.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid AMP, batch no. 17133/90, purity: 96.0 % - 96.3 %
Imidacloprid was tested in the Salmonella/microsome assay at concentrations of up to and including 5000 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-

auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.

Findings:
Imidacloprid AMP concentrations of up to 5000 µg/plate did not cause any mutagenic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed.

Conclusion:
Imidacloprid is considered to be non-mutagenic in this assay with and without metabolic activation.

Report: Herbold, B. (1992)
NTN 33893 AMP W – Salmonella/microsome test.
Bayer AG, unpublished report No.: 21775, date: October 19, 1992

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid AMP W, batch no. 816255007, purity 97.4 %
Imidacloprid was tested in the Salmonella/microsome test at doses up and including 5000 µg/plate in the bacteria strains TA 98, TA 100, TA 535 and TA 1537 with and without metabolic activations. Na-azid, NF, 4-NPDA and 2-AA were used as positive controls. The solvent for all substances was DMSO.

Findings:
There was no evidence for mutagenic effects of imidacloprid AMP W with and without metabolic activation.

Conclusion:
Imidacloprid AMP W is considered to be negative in the Salmonella/microsome test with and without metabolic activation.

Report: Watanabe, M. (1991a)
Reverse mutation assay (Salmonella typhimurium and Escherichia coli).
Nihon Bayer Agrochem K.K., unpublished report No.: RA91002, date: 17.1.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 93.7 %
Imidacloprid was tested for mutagenic effects with and without metabolic activation using Salmonella typhimurium TA98, TA100, TA1535 and TA1537 strains as well as Escherichia coli WP2/uvrA strain up to and including 5000 µg per plate. The solvent was DMSO. 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, 2-Aminoanthracene, N-Ethyl-N'-nitro-N-nitrosoguanidine and 9-Aminoacridine were used as positive controls.

Findings:
Imidacloprid concentrations of up to and including 5000 µg/plate did not produce an increase in the mutant frequency. No bacteriotoxic effect occurred. The positive controls demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid is considered to be non-mutagenic in these assays with and without metabolic activation.

Report: Watanabe, M. (1990a)
NTN 33893 – Rec-assay with spores in the bacterial system.
Nihon Bayer Agrochem K.K., unpublished report No.: RA90016,
date: 18.6.1990

GLP: Yes (certified laboratory). Deviations: none

Guideline: In compliance with MAFF (59 Nohsan No. 4200).

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 94.7 %
Imidacloprid was investigated in the recombination assay with spores from Bacillus subtilis strains H17 (rec+) and M45 (rec-) for DNA-damage up to and including 5000 µg per plate with and without metabolic activation.

Findings:
Imidacloprid concentrations of up to and including 5000 µg/plate did not produce any growth inhibition in both strains, suggesting that no DNA-damage was induced both with and without S-9 activation system. The positive controls demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid did not lead to recombination repair in this test system.

Report: Lehn, H. (1989a)
NTN 33893 – Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro.
Bayer AG, unpublished report No.: 17578, date: 6.1.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 476, FIFRA PB 84-233295, 88/302/EEC.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.2 %
Imidacloprid was tested for mutagenic effects at the HGPRT locus (forward mutation assay) in CHO cell cultures (CHO-K1-BH4) after in vitro treatment at concentrations up to 125 µg/mL without S-9 mix and 1222 µg/mL with S-9 mix. The solvent was DMSO. Ethanemethanesulfonate (EMS) was used as a positive control without S9 mix, and dimethylbenzanthracene (DMBA) as a positive control with S9 mix.

Findings:
Imidacloprid concentrations of up to 125 µg/mL without metabolising enzyme fraction and 1222 µg/mL with S-9 mix did not produce an increase in the mutant frequency. Cytotoxic effects with a relative survival < 50 % were observed at ≥ 90 µg/mL without and at ≥ 800 µg/mL with S-9 mix. The positive controls demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid is considered to be non-mutagenic in the HGPRT test with and without metabolic activation.

Report: Herbold, B. (1988a)
NTN 33893 – Test on *S. cerevisiae* D7 to evaluate for induction of mitotic recombination.
Bayer AG, unpublished report No.: 16832, date: 27.6.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 480; Deviations: none

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.3 %
Imidacloprid was tested for induction of mitotic recombination (gene conversion and crossing-over) using Saccharomyces cerevisiae, strain D7, at concentrations of up to 10000 µg/mL. The solvent was DMSO. Methyl methane sulphonate and cyclophosphamide were used as positive controls.

Findings:
Imidacloprid concentrations of up to and including 10000 µg/mL did not produce an increase in the mutant frequency. Evidence for induction of mitotic recombination by imidacloprid was not found. The positive controls demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid is considered to be non-mutagenic in this test system.

Report:	Cifone, M.A. (1988) Mutagenicity test on NTN 33893 in the rat primary hepatocyte un-scheduled DNA synthesis assay. Hazleton, unpublished report No.: R 4631, date: 21.12.1988
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	OECD 482, FIFRA PB 84-233295, 88/302/EEC.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.2 %,
Imidacloprid was tested for induction of ^{3}H-thymidine incorporation in the in vitro rat primary hepatocyte unscheduled DNA (UDS) assay. Rat primary hepatocytes were exposed to imidacloprid concentrations from about 750 µg/mL to 5.00 µg/mL. The solvent was DMSO. 2-Acetyl aminofluorene was used as the positive control.

Findings:
Imidacloprid did not induce significant changes of nuclear labelling of rat primary hepatocytes in the concentration range tested. The positive control substance demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid is considered not to be a DNA-damaging substance in the rat primary hepatocyte UDS assay.

Report: Taalman (1988)
NTN 33893 – Clastogenic evaluation of NTN 33893 in an in vitro cytogenetic assay measuring sister chromatid exchange in Chinese hamster ovary (CHO) cells.
Hazleton, unpublished report No.: R 4407, date: 21.4.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.2 %
Imidacloprid was tested for clastogenic activity in the in vitro cytogenetic assay measuring sister chromatid exchange in Chinese hamster ovary cells without and with S9-mix up to and including 5000µg/mL. The solvent was DMSO. Mitomycin-C for the non-activation and cyclophosphamide in the metabolic activation series were used as positive controls.

Findings:
A statistically significant increase in SCE-rate at concentrations of 250 – 1000 µg/mL (without S9-mix) and of 2000 – 3000 µg/mL (with S9-mix). Mitotic inhibition and cell cycle delay were observed in the same concentration range.

Conclusion:
Imidacloprid was found to induce weak clastogenic effects in CHO cells in vitro.

Report: Putman, D.J. and Morris, M.J. (1989)
Sister chromatid exchange assay in Chinese hamster ovary cells.
Microbiological Associates Inc., unpublished report No.: BC1149, date: 12.9.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. PF 17001/88, purity: 95.2 %,
Imidacloprid was tested for clastogenic effects in the sister chromatic assay both in the absence and presence of S9-mix activation system at dose levels of 25, 50, 100, 200 and 400

µg/mL in the non-activated study and 157, 313, 625 and 1250 µg/mL in the S9-mix activated study. The solvent was DMSO. Triethylenemelamine and cyclophosphamide were used as the positive controls.

Findings:
Imidacloprid did not induce a relevant increase in sister chromatid exchange without and with S9-mix. The positive controls demonstrated a good sensitivity of this assay.

Conclusion:
Imidacloprid is considered not to induce clastogenic effects in CHO cells in this test.

Report:	Herbold, B. (1989b) NTN 33893 – *In vitro* cytogenetic study with human lymphocytes for the detection of induced clastogenic effect. Bayer AG, unpublished report No.: 18092, date: 16.6.1989 *+ Addendum 18092A, date: 24.8.1989.*
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, main study: batch no. 180587, purity: 95.2 %; addendum: batch no. 880226ELB01, 99.8 %
Imidacloprid was evaluated in the human lymphocyte cytogenetics assay in vitro for clastogenic effects at concentrations of up to 5200 µg/mL without and with S9-mix. The solvent was DMSO. Mitomycin C and cyclophoshamide were used as positive controls.

Findings:
Imidacloprid showed a clastogenic effect without S9-mix at cytotoxic concentrations of 500 – 5200 µg/mL. With S9-mix a weak clastogenic effect could not be ruled out. 50 µg/mL were without any effects. The positive controls had a clear clastogenic effect. In the second study the clastogenic effect of imidacloprid was reproduced . This supports the conclusion that the clastogenic effect was not due to by-products present in the batch used for the first study.

Conclusion:
Imidacloprid is considered to induce clastogenic effects in human lymphocytes *in vitro*.

B.6.4.2 *In vivo* genotoxicity in somatic cells

Report: Herbold, B. (1989c)
NTN 33893 – *In vivo* cytogenetic study of the bone marrow in Chinese hamster to evaluate for induced clastogenic effects.
Bayer AG, unpublished report No.: 18557, date: 24.11.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 475

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 94.6 %
Test animals: Chinese hamster
Imidacloprid was tested for clastogenic effects using the cytogenetic test on bone marrow of Chinese hamster *in vivo* following a single oral treatment of 2000 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and dissolved in deionised water. Administration volume was 10 mL/kg bw. Metaphases were prepared at 6 hrs, 24 hrs and 48 hrs postdose.

Findings:
The treated animals showed no symptoms. External appearance and physical activity were uneffected. However, four animals died due to the acute toxicity of 2000 mg/kg bw. Symptoms of intoxication were not reported for theses animals. No changes indicative of a clastogenic effect of imidacloprid were observed. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
Imidacloprid is found not to induce a clastogenic effect in Chinese hamster bone marrow in vivo.

Report: Herbold, B. (1988b)
NTN 33893 – Micronucleus test on the mouse to evaluate for clastogenic effects.
Bayer AG, unpublished report No.: 16837, date: 27.6.1988

GLP: Yes (certified laboratory). Deviations: none

Guideline: EEC B.12, OECD 474, US EPS 1984 PB 84-23329.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.3 %
Test animals: Mouse (Strain Bor:NMRI (SPF Han), Breeder F. Winkelmann, Borchen, Germany)
Imidacloprid was tested for clastogenic effects in bone marrow with the micronucleus test on the mouse *in vivo* following a single oral administration of 80 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and administered in doses of 20 mg/kg bw. Administration volume was 10 mL/kg bw. Polychromatic erythrocytes were evaluated for micronuclei at 24 hrs, 48 hrs and 72 hrs postdose.

Findings:
Animals treated with imidacloprid showed symptoms of toxicity (apathy, decreased motility, respiratory difficulties) for up to six hours after administration. All animals survived until the end of the study. The ratio of polychromatic to normochromatic erythrocytes was not altered. No indications of a clastogenic effect were found. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
Imidacloprid is found to be non clastogenic in mice bone marrow *in vivo*.

Report: Herbold, B. (1989d)
NTN 33893 – Sister chromatid exchange in bone marrow of Chinese hamster *in vivo*.
Bayer AG, unpublished report No.: 18093, date: 16.6.1989
+ Supplement report No. 18093A, date: 23.11.1993.

GLP: Yes (certified laboratory). Deviations: none

Guideline: In main accordance to OPPTS 870.5915.

Deviations: Only SCE per metaphase established.

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 95.0 %
Test animals: Chinese hamster
Imidacloprid was tested for DNA-modifications using the sister chromatid exchange method in vivo following a single oral treatment of 500, 1000 and 2000 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control, dissolved in deionised water and applied at a single dose of 10 mg/kg bw. Administration volume was 10 mL/kg bw.

Findings:
The treated animals showed no symptoms. All animal survived until the end of the study. Slightly but significant reduction (17 %) of the mitotic index occurred at 1000 mg/kg bw and 2000 mg/kg bw. No changes indicative of a clastogenic effect of imidacloprid were observed. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
Imidacloprid is found not to induce clastogenic effects (sister chromatid exchange) in bone marrow of Chinese hamster *in vivo*.

B.6.4.3 *In vivo* genotoxicity in germ cells

Report:	Völker, W. (1990) Mouse germ-cell cytogenetic assay with NTN 33893. Cytotest Cell Research GmbH & Co. KG (CCR), unpublished report No.: R 5063, date: 22.5.1990
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	OECD 483; EU 87/302EEC.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 180587, purity: 94.1 %
Test animals: male NMRI mice (Breeder BRL Tierfarm, Fuellinsdorf, Switzerland)
Imidacloprid was tested for chromosome aberration by means of the mouse germ-cell cytogenetic assay *in vivo* following a single oral dose of 80 mg/kg bw. Doxorubicin-sulfate hydrochloride was used as positive control and administered intraperitoneal at a single dose of 10 mg/kg bw. Administration volume was 10 mL/kg bw.

Findings:
The test article did not induce chromosome aberrations at the maximum tolerated dose of 80 mg/kg bw. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
Imidacloprid is found to be non-mutagenic in the mouse germ cell chromosome aberration assay.

B.6.5 Long-term toxicity and carcinogenicity (Annex IIA 5.5)

Reduced feed intakes and reduced body weight gains were sensitive endpoints in the long-term toxicity studies performed with imidacloprid. The liver and possibly the CNS turned out to be target organs in mice. Findings such as liver cell hypertrophy and cytochrome P450 activity increase are to be seen as an adaptation process of the organ for a more effective metabolisation of the compound. In contrast, decreased plasma cholesterol levels in rats (observed only in short-term studies) and mice are indicative for liver effects and dysregulation of lipid metabolism.
An increased incidence of mineralisation in the colloid of the thyroid gland follicles was determined in the rat chronic feeding study. The increased incidence of this finding, which occurs as a spontaneous phenomenon in ageing rats (senescent involution of isolated thyroid follicles), is considered a treatment-related effect indicative of a premature biological ageing

process. An effect on thyroid function can be excluded since the plasma levels of thyreotropin, triiodothyronine and thyroxine remained unchanged.
An increased incidence of mineralisation in the thalamus was seen in mice at very high dose levels. Mice in this dose group displayed behavioural abnormalities (increased vocalisation) and an apparent increase in adverse outcomes after ether narcosis for blood withdrawal.
No evidence of an oncogenic potential of imidacloprid was found in neither the rat nor the mouse long-term feeding studies.

Table B.6.5-1: Summary of long-term toxicity and carcinogenicity studies

Type of study	Animal species	Dose range tested	NOEL/NOAEL/ NOEC	Reference
Chronic toxicity and carcinogenicity	Rat	0-100-300-900-1800 ppm	m: 100 ppm (5.7mg/kg bw/day) f: 300 ppm (24.9 mg/kg bw/day)	Eiben & Kaliner, 1991
Carcinogenicity	Mouse	0-100-330-1000-2000 ppm	m/f: 330 ppm (65.6/103.6 mg/kg bw/day)	Watta-Gebert, 1991

B.6.5.1 Rat

Report: Eiben, R. and Kaliner, G. (1991)
NTN 33893 (proposed common name: *Imidacloprid*) – Chronic toxicity and carcinogenicity studies on Wistar rats (administration in food over 24 months).
Bayer AG, unpublished report No.: 19925, date: 25.1.1991
+ Supplement MTD study, unpublished report No. 20541, date: 19.8.1991.

GLP: Yes (certified laboratory), Deviations: none

Guideline: OECD 453, FIFRA § 83-5, EU 88/302/EEC.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, mixed batch no.180587, purity: 94.3 % - 95.3 %
Test animals: Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder Winkelmann, Borchen, Germany)
Imidacloprid was administered to groups of 50 male and 50 female Wistar rats in their diet at concentrations of 0, 100, 300 and 900 ppm for 24 months. In a supplement MTD study, groups of 50 male and female Wistar rats were administered imidacloprid at levels of 0 and 1800 ppm in their diet for 24 months. Ten additional rats per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.

Findings:
General observations: Appearance, behaviour, food intakes and mortality were unaffected in males and females at 1800 ppm. Water intake was reduced by 13 % in females at 1800 ppm. Reduced weight gains were noted in males and females at 900 ppm and above with the decreases amounting to 11 - 12 % at 1800 ppm. The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight.

Haematology, clinical chemistry, urinalysis: The haematological tests gave no indications of haematotoxicity or damage to the haematogenic organs at dose levels up to 1800 ppm. The plasma, erythrocyte and brain cholinesterase activities were not significantly affected; adverse effects on, or functional impairment of any organ could not detected in males and females up to and including 1800 ppm.

Figure B.6.5-1: Main chronic study in rats - Body weights (mean values) - males

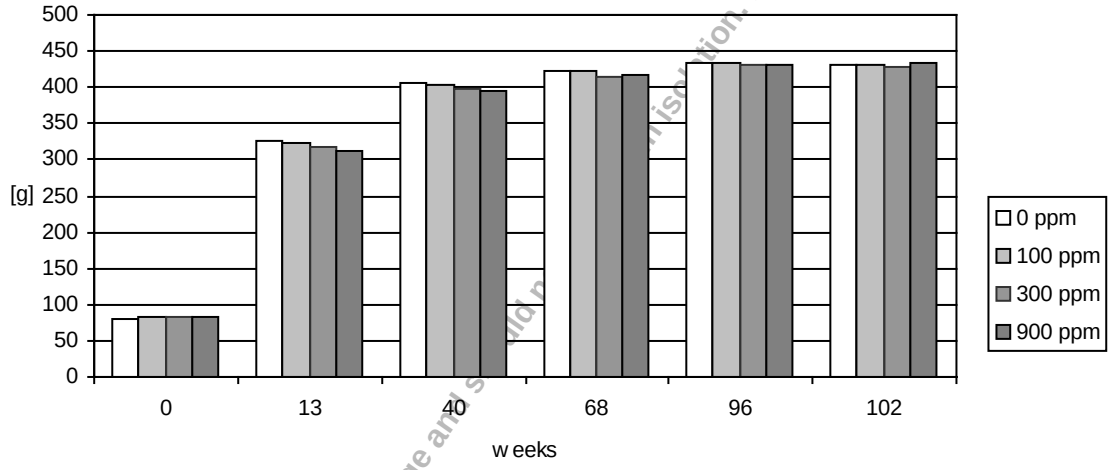


Figure B.6.5-2: Supplement chronic study in rats - Body weights (mean values) - males

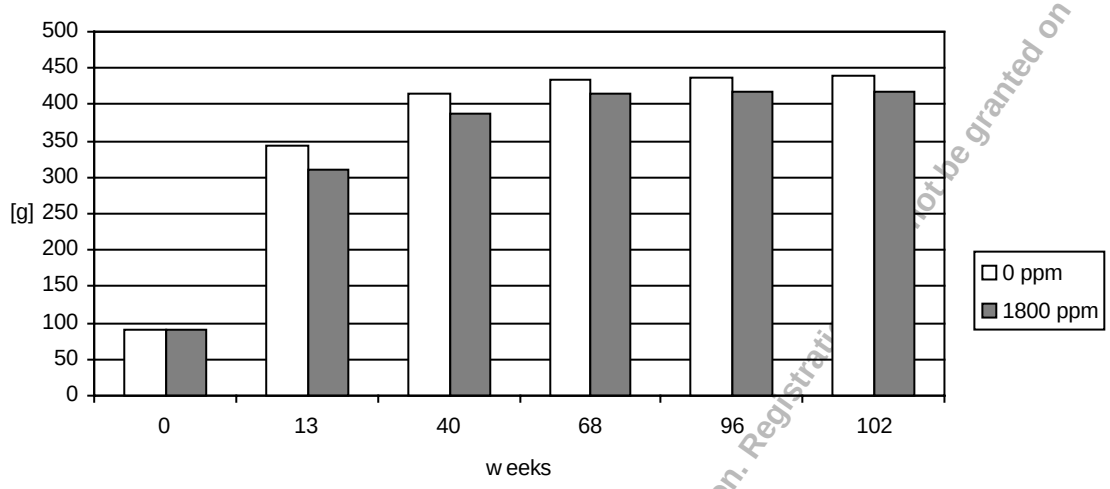


Figure B.6.5-3: Chronic study in rats - Body weights (mean values) - females

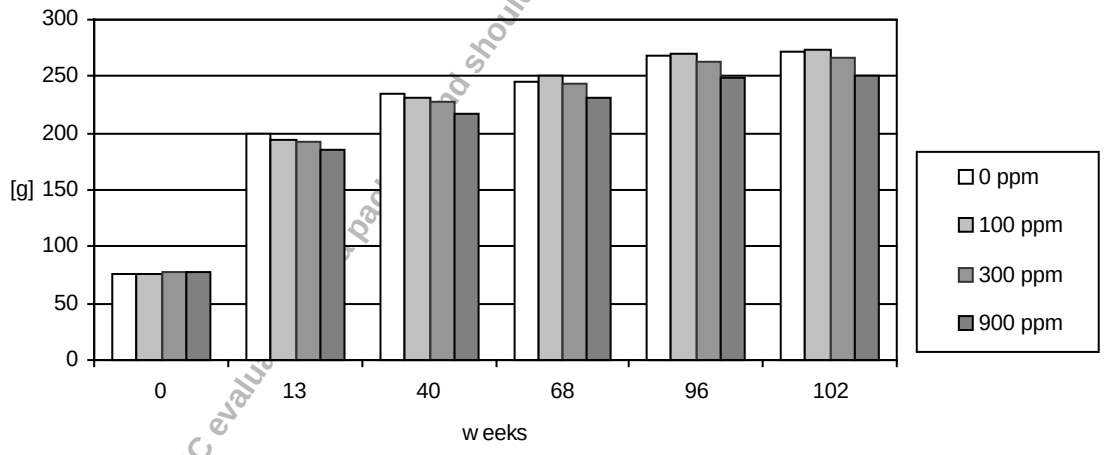


Figure B.6.5-4: Supplement chronic study in rats - Body weights (mean values) - females

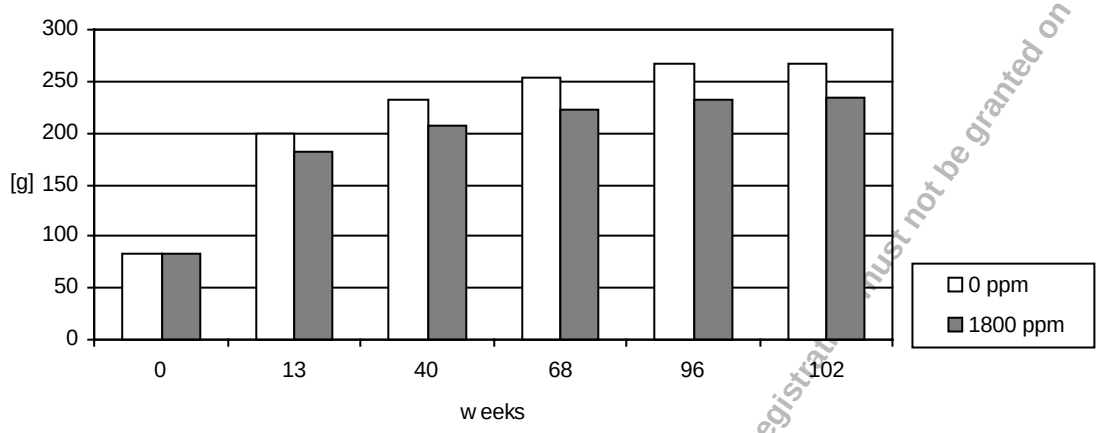


Table B.6.5-2 : Chronic study in rats - Absolute organ weights after 12 months

Dose	0 ppm	100 ppm	300 ppm	900 ppm
Males				
Liver [mg]	14355	14559	13935	12421+
Kidney [mg]	2446	2456	2457	2245
Females				
Liver [mg]	8548	7749	8275	7371+
Kidney [mg]	1629	1549	1543	1419++

+ = $p \leq 0.05$; ++ = $p \leq 0.01$ (Mann-Whitney + Wilcoxon Test, two-sided)

Gross pathology, organ weights, histopathology: Absolute liver and kidney weights were reduced after 12 months in females at 900 ppm; males exhibited lower liver weights at 900 ppm at this time. These deviations from the control values are not attributed to liver or kidney damage, but are seen in relation to the reduced body weight gain in these dose groups. Histopathological assessment of these organs produced no evidence for treatment-related lesions. Increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in males beginning at 300 ppm and in females at 900 ppm. At 1800 ppm fewer colloid aggregations and more parafollicular hyperplasias of minimal intensity were observed. These findings occur spontaneously in ageing rats and indicate involution of isolated follicles related to senescence. In these studies they are regarded as a treatment effect on the thyroid resulting in premature biological ageing processes in this organ. No treatment-related changes in TSH, T3 or T4 were observed after 76 weeks of treatment at 1800 ppm.

The nature, location, incidence and latency periods of the tumors in this study presented no evidence for an oncogenic effect of imidacloprid.

Table B.6.5-3: Chronic study in rats – Histopathological findings

Dose	week	0 ppm	100 ppm	300 ppm	900 ppm	0 ppm	1800 ppm
Males							
Thyroid: mineralised follicular colloid	*52*	3 / 10	3 / 10	6 / 10	**10 / 10**	5 / 10	**10 / 10**
	104	2 / 50	12 / 50	**31 / 50**	**44 / 50**	12 / 50	**46 / 50**
Thyroid: parafollicular cell hyperplasia	*52*					1 / 10	0 / 10
	104	5 / 50	5 / 50	4 / 50	6 / 50	4 / 50	**12 / 50**
Thyroid: colloid aggregation	*52*					6 / 10	**0 / 10**
	104					41 / 50	**20 / 50**
Eye: retinal degeneration/ atrophy	*52*	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10	3 / 10
	104	15 / 50	19 / 50	14 / 50	15 / 50	29 / 48	29 / 49
Harderian gland: porphyrin accummulation	*52*	0 / 10	0 / 10	0 / 10	0 / 10	3 / 10	3 / 10
	104	21 / 50	11 / 50	12 / 50	**6 / 50**	33 / 50	33 / 50
Kidney: nephropathy	*52*	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10	0 / 10
	104	29 / 50	30 / 50	25 / 50	21 / 50	29 / 50	**9 / 50**
Females							
Thyroid: mineralised follicular colloid	*52*	0 / 10	0 / 10	0 / 10	3 / 10	2 / 10	**5 / 10**
	104	11 / 50	6 / 50	11 / 50	**27 / 50**	3 / 50	**38 / 50**
Thyroid: parafollicular cell hyperplasia	*52*					0 / 10	2 / 10
	104	5 / 50	5 / 50	10 / 50	5 / 50	5 / 50	8 / 50
Thyroid: colloid aggregation	*52*					2 / 10	**0 / 10**
	104					22 / 50	**7 / 50**
Eye: retinal degeneration/ atrophy	*52*	0 / 10	0 / 10	0 / 10	0 / 10	4 / 10	3 / 10
	104	17 / 50	25 / 50	10 / 50	23 / 50	27 / 50	**39 / 50**
Harderian gland: porphyrin accummulation	*52*	0 / 10	0 / 10	0 / 10	0 / 10	3 / 10	0 / 10
	104	0 / 50	7 / 50	3 / 50	2 / 50	17 / 50	**28 / 50**
Kidney: nephropathy	*52*	0 / 10	0 / 10	0 / 10	0 / 10	1 / 10	0 / 10
	104	18 / 50	9 / 50	**3 / 50**	**5 / 50**	12 / 50	**1 / 49**

Conclusion:
NOAEL: 100/300 ppm (males/females), equivalent of 5.7 mg/kg bw/day for males and 24.9 mg/kg bw/day for females based on thyroid effects (increased incidence of colloid mineralisation) in males and females at 300 ppm and 900 ppm, respectively and on reduced body weight gains in males and females at 900 ppm.

B.6.5.2 Mouse

Report: Watta-Gebert, B. (1991)
NTN 33893 (proposed common name *imidacloprid*) –
Carcinogenicity study on B6C3F1 mice (administration in the food for 24 months).
Bayer AG, unpublished report No.: 19931, date: 28.1.1991
+ Supplementary MTD study, unpublished report No.: 20769, date: 24.10.1991.

GLP: Yes (certified laboratory), Deviations: none

Guideline: In compliance with OECD 451, FIFRA, § 83-2. Deviations: none

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, mixed batch no.180587, purity: 95.3 %; supplementary study: 95.0 %;
Test animals: B6C3F1 mice (Breeder Charles River Wiga, Sulzfeld, Germany)
Imidacloprid was administered to groups of 50 male and 50 female mice in their diet at concentrations of 0, 100, 330 and 1000 ppm for 24 months. In a supplement MTD study, groups of 50 male and female mice were administered imidacloprid at levels of 0 and 2000 ppm in their diet for 24 months. Ten additional mice per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was 20.2, 65.6, 208.2 or 413.5 mg for males and 30.3, 103.6, 274.4 or 423.9 mg for females.

Findings:
General observations: Unusual vocalisations, squeaking and twittering, which increased whenever the animals became agitated were observed throughout the study in males and females at 2000 ppm. In addition, hypersensitivity to ether narcosis and/or blood withdrawal resulting in increased mortality after manipulations was observed in this dose group. Nine males and four females died after anaesthesia for tattooing or blood sampling compared with no mortalities in the control group. Similar observations were made in the 15-week range-finding study (see B.6.3.2.2). Food intake was slightly reduced in females at 1000 ppm and markedly reduced in females (-24 %) at 2000 ppm. Water intake was decreased slightly in females at 1000 ppm and markedly in males (-11 %) and females (-27 %) at 2000 ppm. Body weight development was not influenced at doses up to and including 330 ppm. At 1000 ppm the mice exhibited reduced weight gain and marked reductions were seen at 2000 ppm (up to -29 % in males and -26 % in females).

Table B.6.5-4: Supplement chronic study in mice - Food and water intake

Dose	0 ppm	100 ppm	330 ppm	1000 ppm	0 ppm	2000 ppm
Males						
Food intake [g/kg bw/day]	203.6	202.1	198.7	208.2	192.4	206.8
Water intake [g/kg bw/day]	183.7	187.8	189.0	186.6	189.2	169.1
Females						
Food intake [g/kg bw/day]	296.1	302.9	314.0	274.4	280.3	212.0
Water intake [g/kg bw/day]	235.5	231.0	236.5	210.9	246.6	180.7

Table B.6.5-5: Chronic study in mice – Mean body weights

Dose	0 ppm	100 ppm	330 ppm	1000 ppm	0 ppm	2000 ppm
Males						
Week 0	20.7	20.3	19.9	20.3	24	25
Week 13	27.8	27.5	27.1++	26.7++	31	27++
Week 27	30.4	30.3	29.4++	28.9++	34	29++
Week 41	31.8	30.9	31.1	29.7++	37	30++
Week 55	32.9	32.2	31.6+	31.1++	40	30++
Week 69	33.6	33.5	32.8	31.7++	40	30++
Week 83	33.9	33.6	33.6	32.5+	40	30++
Week 97	34.5	33.4	34.1	32.9+	39	30++
Week 104	33.4	32.7	33.4	32.5	40	30++
Females						
Week 0	16.3	15.8	15.6	16.0	21	21
Week 13	24.4	24.1	23.9	24.5	27	24++
Week 27	26.6	26.1	26.0	26.3	29	25++
Week 41	27.4	27.4	27.2	27.1	30	26++
Week 55	28.1	27.9	27.7	28.2	32	26++
Week 69	29.5	28.9	28.6	29.1	34	27++
Week 83	29.2	29.2	28.5	28.9	34	27++
Week 97	29.5	29.2	29.1	29.0	32	27++
Week 104	29.3	29.2	28.8	29.1	34	27++

+ $p \leq 0.05$; ++ $p \leq 0.01$ (Mann-Whitney U-Test, two-tailed)

Haematology, clinical chemistry, urinalysis: No indications of treatment-related haemotoxicity or damage to the haematogenic organs was found at 1000 ppm. At 2000 ppm lower leukocyte counts were determined in both sexes. The clinical chemistry gave no evidence for liver damage. Reduced blood cholesterol levels at 2000 ppm indicate an effect on the lipid metabolism in this group. Kidney-related clinical chemistry parameters in the blood were unaffected in the 2000 ppm group.

Table B.6.5-6: Chronic study in mice – Haematology and clinical chemistry

Dose	week	0 ppm	100 ppm	330 ppm	1000 ppm	0 ppm	2000 ppm
Males							
leuco [10^9/L]	*52*	4.6	4.6	5.1	4.3	5.7	4.3*
	103/102	4.5	5.7*	5.8	5.4	7.0	5.3
chol [mmol/L]	*54*	3.14	3.14	2.97	3.11	3.54	2.55**
	104	3.37	3.75	3.91	3.62	4.29	2.85**
Females							
leuco [10^9/L]	*52*	4.1	4.3	4.5	3.8	4.6	2.9**
	103/102	7.7	3.5	4.1	3.2*	5.5	3.9
chol [mmol/L]	*54*	2.53	2.48	2.56	2.52	2.40	2.13*
	104	2.56	2.26	3.07	2.45	2.58	2.34

* $p \leq 0.05$; ** $p \leq 0.01$ (Mann-Whitney U-Test, two-tailed)

Gross pathology, organ weights, histopathology: Morphological evidence for a marginal functional effect on the liver (low-grade periacinal hepatic cell hypertrophy) was found in a few males at 2000 ppm. This marginal change probably results from hepatocyte adaptation to the foreign substance metabolism, and should not be interpreted as evidence for liver damage. At 2000 ppm more animals than in the control groups exhibited mineralisation of the thalamic region of the brain. Unfortunately, the region of brain mineralisation is only identified in the report on the MTD study but not in the main study, so that a direct comparison with the findings of the first study is not possible.
The nature, location, incidence and latency periods of the detected tumors presented no evidence for an oncogenic effect.

Table B.6.5-7 Chronic study in mice – Histopathological findings

Dose	week	0 ppm	100 ppm	330 ppm	1000 ppm	0 ppm	2000 ppm
Males							
Liver: Periacinal cell hypertrophy	*104*	0 / 50	0 / 50	0 / 50	0 / 50	0 / 50	**5 / 49**
Brain: Thalamus mineralisation	*104*	22 / 50 (brain)	25 / 50 (brain)	24 / 50 (brain)	15 / 50 (brain)	17 / 50 (thalamus)	**24 / 50 (thalamus)**
Females							
Liver: Periacinal cell hypertrophy	*104*	0 / 50	0 / 50	0 / 50	0 / 50	0 / 49	0 / 49
Brain: Thalamus mineralisation	*104*	12 / 50 (brain)	26 / 50 (brain)	21 / 50 (brain)	8 / 50 (brain)	14 / 50 (thalamus)	**24 / 50 (thalamus)**

Conclusion:
NOAEL: 330 ppm (males/females), equivalent to 65.5 mg/kg bw/day for males and 103.6 mg/kg bw/day for females based on reduced body weights at 1000 ppm.

B.6.6 Reproductive toxicity (Annex IIA 5.6)

The reproductive toxicity of imidacloprid was investigated in a two-generation study in rats and in developmental toxicity studies in rats and rabbits. Reproduction behaviour and outcome was not negatively affected by the treatment with imidacloprid. Reduced body weight gain was the most sensitive parameter in parents and pups.
Effects on body weight gain as a consequence of reduced food consumption were observed in the rat and rabbit developmental toxicity studies. A slightly increased incidence of wavy ribs

was the only developmental effect established in rats, observed only at a dose which produced maternal toxicity as well. In rabbits, total litter losses and a slight decrease in foetal body weight with retarded ossification were found in the maternally toxic dose range only.
Overall the data show that imidacloprid has no primary reproductive toxicity and exerts no teratogenic potential.

Table B.6.6-1: Summary of reproductive toxicity studies

Type of study	Animal species	Dose range tested	NOEL/NOAEL	Reference
1-Generation	Rat	0-20-100-500 ppm	adult: m & f: 100 ppm (9 mg/kg bw/day) reproduction: m & f: 500 ppm (40 mg/kg bw/day) development: 100 ppm (8 - 20 mg/kg bw/day)	Suter et al., 1990
2-Generation	Rat	0-100-250-700 ppm	adult: m & f: 250 ppm (~ 20 mg/kg bw/day) reproduction: m & f: 700 ppm (~ 50 mg/kg bw/day) development: 250 ppm (~ 40 mg/kg bw/day)	Suter et al., 1990
Embryotoxicity	Rat	0-10-30-100 mg/kg bw/day	maternal: 30 mg/kg bw/day development: 30 mg/kg bw/day	Becker et al., 1988a
Embryotoxicity	Rabbit	0-8-24-72 mg/kg bw/day	maternal: 8 mg/kg bw/day development: 24 mg/kg bw/day	Becker et al., 1988b

B.6.6.1 Multi-generation studies in rats

Report: Suter, P., Vogel, W., Wilson, Th. and Terrier, Ch. (1990) NTN 33893 technical – Range finding study to the multiple generation study in rats. [redacted] unpublished report No.: R 4955, date: 23.2.1990

GLP: No

Guideline: Not applicable (dose range finding study)

Deviations: Not applicable

Acceptability: The study is considered to be acceptable for dose-range finding.

Material and Methods:
Test material: Imidacloprid technical, batch no. 2/86, purity 92.8 %
Test animals: Wistar/HAN rats (Strain Strain Kfm:WIST; Breeder KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland)
To determine the suitable dose levels for a subsequent 2-generation reproduction study imidacloprid was mixed with feed at concentrations of 0, 20, 100 and 500 ppm and offered to male and female animals from the P generations during a three-week premating period and throughout the pairing, pregnancy and lactation periods. Each group comprised 10 male and 10 female rats. Litters were reduced to 8 pups on postnatal day 4. Following the weaning of

F1 litters on day 21 post partum, the pups were reared for a further week on the respective test diet. Substance intake for P generation males was 0, 2, 9 and 40 - 48 mg/kg bw/day during the premating period. Doses for P generation females amounted to 0, 2, 8 - 10 and 37 - 51 mg/kg bw/day during premating period and pregnancy and to 0, 3 - 4, 14 - 20 and 70 - 95 mg/kg bw/day during the lactation period.

Table B.6.6-2: One-generation study in rats - Test substance intake

	Test substance intake (mg/kg bw/d)		
Feed concentration:	**20 ppm**	**100 ppm**	**500 ppm**
Males	2	9	40-48
Females pre-mating	2	8-10	38-51
Females pregnancy	2	8-9	37-44
Females lactation*	3-4	14-20	70-95

* until day 14 postpartum

Findings:
General observations: Viability, behaviour and general appearance of all parents and F1 pups were not influenced by treatment up to and including 500 ppm. At 500 ppm slightly decreased mean food consumption and body weight gain was observed in the parent females, mainly during the premating period. Pups of the dams treated with 500 ppm had decreased mean body weights at birth and displayed lower postnatal growth and mean food consumption after weaning.
Reproductive toxicity: There were no treatment-related changes in mating performance and fertility, precoital time, percentage of mating, fertility index, conception rate, pregnancy index, mean number of implantation sites and post-implantation loss. No changes were observed with regard to duration of pregnancy, parturition and nursing, mean number of pups per dam, perinatal and postnatal pup mortality.
Gross necropsy, organ weights, histopathology: There were no treatment-related changes in the absolute and relative organ weights in any dose group or sex. No macroscopic abnormal findings were noted in any parent animal or F1 pup up to 500 ppm. Histopathological examination performed on liver, testes and ovaries of parent animals showed no evidence of abnormal findings resulting from treatment with imidacloprid. No teratogenic effect of imidacloprid was noted in any dose group.

Conclusion:
NOAEL parental: 100 ppm, equivalent to approximately 9 mg/kg bw/day during the premating period, based on reduced food consumption and body weight gain in females at 500 ppm.
NOAEL reproduction: 500 ppm, equivalent to approximately 40 - 50, 37 - 44 and 70 - 95 mg/kg bw/day during premating period, pregnancy and lactation, respectively
NOAEL development: 100 ppm, equivalent to approximately 9 mg/kg bw/day during pregnancy and 14 - 20 mg/kg bw/day during lactation, based on reduced birth weights and body weight gains in pups at 500 ppm

Report: Suter, P., Biedermann, K., Luetkemeier, H., Wilson, J.Th. and Terrier, Ch. (1990)
NTN 33893 technical (proposed c.n. imidacloprid) – Multiple generation reproduction study in rats.
[redacted] unpublished report No.: R 5097, date: 21.6.1990
+ Amendment, unpublished report Nos.: R 5097 A, date: 21.11.1990; R 5097 B, date: 3.3.1992.

GLP: Yes (certified laboratory); Deviations: No QAU inspections of analytical work and triglyceride determinations

Guideline: OECD 416; FIFRA §83-4; Guidance on Toxicology Data No. 4200, Japan.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid technical, mixed batch no. 180587, purity 94.4 % - 95.3 %
Test animals: Wistar/HAN rats (Strain Kfm:WIST; Breeder KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland)
Imidacloprid was administered to Wistar rats in the diet at concentrations of 0, 100, 250 and 700 ppm during a 84 day premating period and throughout the mating, pregnancy and lactation periods for breeding of the F1A and F1B litters. Following weaning of the F1B litters on day 21 post partum, the F1 generation parent animals were selected. The treated diets were fed to F1 parents for 105 days prior to breeding of the F2A litters. The study was terminated after weaning of the F2B litters. Each group of the P parent generation consisted of 30 male and 30 female rats and each group of the F1 parent generation consisted of 26 male and 26 female rats.

Table B.6.6-3: Two-generation study in rats – Test substance intake

	Test substance intake (mg/kg bw/d)					
Feed concentration:	**100 ppm**		**250 ppm**		**700 ppm**	
Generation:	**F0**	**F1**	**F0**	**F1**	**F0**	**F1**
Males	5-8	5-9	14-20	13-23	40-55	36-68
Females (pre-mating)	7-13	6-9	17-30	16-24	48-90	45-68
Females pregnancy (A)	8	7	19	18	53	50
Females lactation* (A)	14	15	38	34	103	102
Females pregnancy (B)	7	7	17	17	46	47
Females lactation* (B)	14	13	36	33	97	96

* until day 14 postpartum; (A), (B) identification of litter

Findings:
Parental toxicity
General observations: Appearance, behaviour and mortality of the parents were unaffected in all treated groups. At 700 ppm reduced food consumption and lower body weight gains were observed in P generation males and females; the food intakes of F1 generation females were decreased.

Table B.6.6-4: Two-generation study in rats - Parental findings (P-generation)

FINDING	Dose level (ppm)			
	0	**100**	**250**	**700**
P Males				
Food consumption, premating [g/d]	23.4	23.5	22.8	22.0+
Body weight gain before mating [g]	248	255	234	222+
P Females				
Food consumption, premating [g/d]	16.7	16.5	16.9	15.9+
Food consumption, pregnancy [g/d] (A)	20.6	20.1	20.8	19.0+
Food consumption, lactation [g/d] (A)	40.4	39.0	43.0	36.4+
Body weight gain before mating [g]	118	115	119	104+
Body weight gain, pregnancy [g] (A)	103	102	109	100
Body weight gain, lactation [g] (A)	32	29	37	28
Producing live litter (A)	29 / 29	28 / 29	28 / 30	29 / 30
Producing live litter (B)	27 / 29	27 / 29	28 / 30	27 / 30

+ $p \leq 0.05$ % (Dunnett test based on pooled variance); (A), (B) identification of litter

Table B.6.6-5: 2- generation study in rats - Parental findings (F1-generation)

FINDING	Dose level (ppm)			
	0	**100**	**250**	**700**
F1 Males				
Food consumption, premating [g/d]	23.8	24.9	24.2	23.6
Body weight gain before mating [g]	198	211	203	197

FINDING	Dose level (ppm)			
	0	100	250	700
F1 Females				
Food consumption, premating [g/d]	17.4	16.8	17.2	16.0+
Food consumption, pregnancy [g/d] (A)	21.8	20.7	21.7	19.0+
Food consumption, lactation [g/d] (A)	39.2	41.4	38.7	37.5
Body weight gain before mating [g]	88	87	86	79
Body weight gain, pregnancy [g] (A)	96	93	94	87
Body weight gain, lactation [g] (A)	26	34	32	36
Producing live litter (A)	22 / 26	23 / 26	22 / 26	25 / 26
Producing live litter (B)	24 / 26	20 / 26	26 / 26	26 / 26

+ $p \leq 0.05$ % (Dunnett test based on pooled variance); (A), (B) identification of litter

Haematology, clinical chemistry, urinalysis: There were no treatment-related effects on haematological parameters of the F1 parents. Elevated cytochrome P-450 and N-demethylase values were determined in males at 700 ppm dose level, and increased O-demethylase activities in males and females. Elevated O-demethylase values were also found in the F1 females at 250 ppm.

Table B.6.6-6: Two-generation study in rats – Clinical chemistry (F1 parents)

FINDING	Dose level (ppm)			
	0	100	250	700
Males				
Cyt. P-450 [nmol/g]	29.3	31.1	29.5	36.8++
N-Demethylase [nmol/min/g]	326.2	338.7	317.6	385.5+
O-Demethylase [nmol/min/g]	8.26	8.36	7.84	11.20++
Females				
Cyt. P-450 [nmol/g]	18.9	20.6	21.1	19.0
N-Demethylase [nmol/min/g]	163.6	142.7	122.3++	152.9
O-Demethylase [nmol/min/g]	6.91	7.49	8.51++	9.47++

+ $p \leq 0.05$ %; ++ $p \leq 0.01$ % (Dunnett test based on pooled variance)

Gross necropsy, organ weights, histopathology: No gross pathological, organogravimetric or histopathological alterations were apparent in the examined parents at doses up to and including 700 ppm.

Reproduction parameters: No treatment-related effects were observed. The following parameters were unaffected: Mean precoital time, fertility and pregnancy indices, conception rate, duration of pregnancy, mean number of viable and stillborn pups per litter, postnatal pup losses up to day 4, and lactation losses up to day 21 after birth.

Table B.6.6-7: Two-generation study in rats - Offspring findings (F1-generation)

FINDING	Dose level (ppm)			
	0	**100**	**250**	**700**
F1A litters				
Live litters	29	28	28	29
Mean litter size	10.7	10.5	11.6	10.7
Mean body weight at birth [g]	5.5	5.6	5.6	5.6
Mean body weight at weaning [g]	47.1	45.5+	46.4	40.8+
F1B litters				
Live litters	27	27	28	27
Mean litter size	11.9	11.2	11.2	10.5
Mean body weight at birth [g]	5.6	5.8	5.8	5.8
Mean body weight at weaning [g]	49.8	50.2	49.7	45.0+

+ $p \leq 0.05$ % (Dunnett test based on pooled variance)

Table B.6.6-8: Two-generation study in rats - Offspring findings (F2-generation)

FINDING	Dose level (ppm)			
	0	**100**	**250**	**700**
F2A litters				
Live litters	22	23	22	25
Mean litter size	10.1	11.0	9.4	9.6
Mean body weight at birth [g]	5.8	5.6	5.7	5.7
Mean body weight at weaning [g]	44.3	44.3	43.6	40.3+
F2B litters				
Live litters	24	20	26	26
Mean litter size	10.8	10.1	9.2	10.7
Mean body weight at birth [g]	5.9	5.8	5.5	5.3
Mean body weight at weaning [g]	50.7	50.5	48.7+	46.0+

+ $p \leq 0.05$ % (Dunnett test based on pooled variance)

Neonatal effects
General observations: Reduced body weight gains were observed at the 700 ppm dose level in pups of the F1 litters and the F2A litters. F2B offspring had lower body weights at birth at the 250 and 700 ppm dose level despite similar or lower mean litter sizes. Reduced growth during the suckling period was present in the 700 ppm group, while pups from the 250 ppm group did to some extent make up for their weight deficiency until weaning.

Gross necropsy, organ weights, histopathology: No gross pathological, organogravimetric or histopathological alterations were apparent in the examined pups at doses up to and including 700 ppm. No teratogenic effect was observed by external examination of the pups in any group of either generation.

Conclusion:
NOAEL parental: 250 ppm, equivalent to approximately 20 mg/kg bw/day during the premating period, based on reduced body weight gain at 700 ppm.
NOAEL reproduction: 700 ppm, equivalent to approximately 50 mg/kg bw/day during premating period and pregnancy
NOAEL development: 250 ppm, equivalent to approximately 40 mg/kg bw/day during lactation, based on reduced body weight gains in pups at 700 ppm

B.6.6.2 Developmental toxicity

B.6.6.2.1 Rat

Report: Becker, H., Vogel, W. and Terrier, Ch. (1988a)
Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rat.
[redacted] unpublished report No.: R 4582, date: 24.11.1988

GLP: Yes (certified laboratory); Deviations: none

Guideline: OECD 414; EPA FIFRA §83-3.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. PT. 17001/87, purity: 94.2 %
Test animals: female Wistar/HAN rats (Strain Kfm:WIST; Breeder KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland)
Imidacloprid was administered to groups of 25 mated female Wistar rats orally by gavage from day 6 through to day 15 post coitum at doses of 0, 10, 30 or 100 mg/kg bw/day in 0.5 % aqueous Cremophor suspension. A standard dose volume of 10 mL/kg bw with daily adjustment to the actual body weight was used. Females were sacrificed on day 21 p.c. and the foetuses were removed by Caesarean section

Findings:
Maternal toxicity: Appearance, behaviour and mortality of the dams were unchanged up to 100 mg/kg bw/day. At 100 mg/kg bw/day the dams showed initial body weight loss and food consumption and body weight gains were reduced during the treatment period. No treatment-related changes were observed at necropsy.

Developmental toxicity: At the highest dose tested no treatment-related changes were observed in the reproduction parameters (incidence of pregnant females and of females with viable foetuses, rates of implantation, viable foetuses, resorptions, mean foetal weights per litter, ratio of male to female foetuses).
No treatment-related changes were determined from external and visceral examination of the foetuses. In the skeletal examination, a slightly increased incidence of wavy ribs (reversible alteration in shape) was observed at 100 mg/kg bw/day. Thus, embryotoxicity of imidacloprid is observed only at a dose which induces moderate maternal toxicity.

Table B.6.6-9: Rat developmental toxicity - Maternal data

FINDING	Dose level (mg/kg bw/d)			
	0	10	30	100
Not pregnant	0 / 25	0 / 25	1 / 25	0 / 25
Live litters at sacrifice	25	25	24	25
Food consumption, Days 6–16 p.c. [g/d]	22.4	22.2	21.0	16.3+
Food consumption, Days 16–21 p.c. [g/d]	22.9	25.2++	24.2	27.6++
Body weight gain, Days 6-16 p.c. [g]	47	45	42	27
Terminal body weight [g]	328	338	325	316
Gravid uterus weight [g]	80.5	81.5	81.4	77.9
Mean corrected weight gain [%]	7.9	8.6	5.6	4.2

+ $p \leq 0.05$ %; ++ $p \leq 0.01$ % (Dunnett test based on pooled variance)

Table B.6.6-10: Rat developmental toxicity - Litter data

FINDING	Dose level (mg/kg bw/d)			
	0	10	30	100
Corpora lutea/dam	14.6	14.8	14.7	13.7
Implantations/dam	13.6	13.3	13.3	12.7
Dams with >2 preimplantation losses	2	6	5	3
Dams with >2 postimplantation losses	2	1	1	0
Mean live litter size	12.6	12.5	12.7	11.9
% males	51	50	51	59
Foetal weight [g]	4.8	4.8	4.8	4.9
Abnormalities [litters/foetuses]	0	1/1	0	0
Wavy ribs [litters/foetuses]	1/2	1/1	0	5/7

Conclusion:
NOAEL maternal: 30 mg/kg bw/day based on reduced body weight gain and reduced food consumption at 100 mg/kg bw/day.
NOAEL developmental: 30 mg/kg bw/day based on increased incidence of wavy ribs at 100 mg/kg bw/day.

B.6.6.2.2 Rabbit

Report: Becker, H., Vogel, W. and Terrier, Ch. (1988b)
Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rabbit.
RCC, unpublished report No.: R 4583, date: 24.11.1988

GLP: Yes (certified laboratory); Deviations: none

Guideline: OECD 414; EPA FIFRA §83-3.

Deviations: No examination of foetal heads for skeletal changes

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. PT 17001/87; purity: 94.2 %
Test animals: female chinchilla rabbits (Strain CHbb:CH hybrids; Breeder KFM, Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland)
Imidacloprid was administered to groups of 16 female chinchilla rabbits orally by gavage at doses of 0, 8, 24 or 72 mg/kg bw/day from day 6 through to day 18 p.c. in 0.5 % aqueous Cremophor suspension. A standard volume of 4 mL/kg bw with daily adjustment to the actual body weight was used. Females were sacrificed on day 28 p.c. and the foetuses were removed by Caesarean section.

Findings:
Maternal toxicity: Decreased body weight gains were found at 24 mg/kg bw/day and higher. Body weight loss from the start of treatment until day 20 p.c. decreased food consumption during the treatment period and mortality were observed at 72 mg/kg bw/day. Two females died on days 18 and 19 p.c., at the end of the treatment period. A further female from this group aborted on day 26 post coitum and two females showed total litter resorption at terminal necropsy.

Developmental toxicity: The body weights of the foetuses were slightly reduced (although the difference did not reach statistical significance) and the incidence of foetuses with retarded ossification was increased at 72 mg/kg bw/day. Because of the reduced litter size in this group which would have resulted in increased foetal weights had there not been foetal toxicity, the reduced foetal weights and the skeletal changes are regarded as signs of foetal retardation and may have resulted from the severe maternal toxicity. No treatment-related malformation were observed.

Table B.6.6-11: Rabbit developmental toxicity - Maternal toxicity

FINDING	Dose level (mg/kg bw/d)			
	0	**8**	**24**	**72**
Found dead (indications of misgavaging)	0/16	0/16	0/16	2/16
Abortion	0 / 16	0 / 16	0 / 16	1 / 14
Total litter resorption	0 / 16	0 / 16	0 / 16	2 / 13
Live litters at sacrifice	16	16	16	11
Food consumption, Days 6–19 p.c. [g/d]	199	197	188	87
Food consumption, Days 19–28 p.c. [g/d]	137	151	168	187
Body weight gain, Days 6-16 p.c. [g]	147	141	99	-173
Terminal body weight [g]	3740	3690	3597	3523
Gravid uterus weight [g]	446	447	420	380
Mean corrected weight gain [%]	-5.9	-6.5	-6.0	-8.2

Table B.6.6-12 Rabbit developmental toxicity - Litter data

FINDING	Dose level (mg/kg bw/d)			
	0	**8**	**24**	**74**
Corpora lutea/dam	9.3	9.4	8.9	9.5
Implantations/dam	8.9	9.0	8.1	8.5
Dams with >2 preimplantation losses	1	0	1	2
Dams with >2 postimplantation losses	0	1	0	1
Mean live litter size	8.5	8.6	7.9	7.5
% males	47	58	48	57
Foetal weight [g]	34.5	32.4	34.0	31.3
Litters with severely weight-retarded foetuses	0	1	0	2
Abnormalities [litters/foetuses]	1/1	1/1	1/1	2/3
Skeletal findings	-	-	-	ossification ↓

Conclusion:
NOAEL maternal: 8 mg/kg bw/day based on reduced body weight gain at 24 mg/kg bw/day.
NOAEL developmental: 24 mg/kg bw/day based on reduced body weights of foetuses and retarded ossification at 72 mg/kg bw/day.

B.6.7 Delayed neurotoxicity (Annex IIA 5.7)

B.6.7.1 Neurotoxicity screening studies in rats

In an acute, a subchronic and a developmental neurotoxicity screening study in rats investigating specific neurotoxicological parameters by a functional observation battery, automated motor activity measurements and special neurohistopathology, behavioural changes in the acute experiment and to a much lesser extent in the developmental study were the only signs that could be indicative of neurotoxic effects. Most clinical signs appeared related to acute receptor-mediated cholinergic toxicity of this chloronicotinyl compound.

Table B.6.7-1: Summary of neurotoxicity studies

Type of study	Animal species	Dose range tested	NOEL/NOAEL	Reference
Acute oral neurotoxicity	Rat	0-20-42-151-307 mg/kg bw	general: 42 mg/kg bw neurotoxicity: 42 mg/kg bw	Sheets & Hamilton., 1994a
Subchronic oral neurotoxicity	Rat	0-140-963-3027 ppm	general: m / f: 9.3 / 10.5 mg/kg bw (140 ppm) neurotoxicity: m / f: 196 / 213 mg/kg bw (3027 ppm)	Sheets & Hamilton., 1994b
Developmental neurotoxicity	Rat	0-95.5-227-691 ppm	maternal: 56 mg/kg bw/day (691 ppm) development: 30 mg/kg bw/day (227 ppm) neurotoxicity: 80 mg/kg bw/day (691 ppm)	Sheets, 2001

B.6.7.1.1 Acute oral

Report: Sheets, L.P. and Hamilton, B.F. (1994a)
An acute oral neurotoxicity screening study with technical grade imidacloprid (NTN 33893) in rats.
Miles Incorporation, unpublished report No.: BC7221, date: 16.2.1994
+ Supplement report No.: BC7221, date: 7.6.1994.

GLP: Yes (certified laboratory)

Guideline: EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 2030030, purity: 97.6 % - 98.8 %
Test animals: Sprague Dawley rats (Strain Sas:CD(SD)BR; Breeder Sasco, Inc., St. Louis, MO, USA)
Imidacloprid was administered by gavage in a single dose to fasted Sprague Dawley rats (18/sex/dose level), using analytically confirmed doses of 0 (vehicle), 42, 151 and 307 mg/kg bw for males and females. In a supplement study imidacloprid was administered to female rats (12/dose) by gavage at analytically confirmed doses of 0 and 20 mg/kg bw. The test substance was suspended in 0.5 % (w/v) methylcellulose with 0.4 % (w/v) Tween 80 in deionised water and administered at a dosing volume of 10 mL/kg bw. Functional observations and tests were conducted on 12 animals/sex/dose level before treatment, on the day of treatment (day 0) and on days 7 and 14 postdose. Behavioural tests on treatment day 0 started at times of peak plasma concentrations. The following observations and measurements were performed in the study: clinical observations, mortality, body weight, automated measurements of activity (figure-eight maze), a functional observational battery, determination of brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from central nervous system were examined histopathologically.

Findings:
General observation: Four high-dose males and ten high-dose females died, either on the day of treatment or within the day following treatment. These deaths were attributed to treatment with imidacloprid. A dose-related increase in the incidence and severity of clinical signs was apparent in males that received 151 or 307 mg/kg of imidacloprid and in females that received the high dose. For males that received the 151 mg/kg dose, this was limited to tremors and nasal stain. The high-dose males had tremors and nasal stain as well as uncoordinated gait, decreased activity, urine stain, and decreased body temperature. Treatment-related effects in high-dose females consisted of tremors, uncoordinated gait, decreased activity, increased reactivity, red nasal stain and decreased body temperature. Clinical signs of toxicity were generally observed on day 0 and resolved in surviving males and females within one to five days following treatment. Body weight was not affected by treatment in surviving males and females.

Functional observational battery (FOB), motor and locomotor activity (MA): In addition to the clinical signs observed in mid and high dose males and high dose females, animals treated with 307 mg/kg bw showed diminished grip strength on treatment day 0. On day 0 a dose-related decrease in motor and locomotor activity was observed in males and females at 151 mg/kg bw and 307 mg/kg bw; seven days after treatment all effects on activity had resolved. Activity data were highly variable; motor activity in individual males and females from the control group on the day of treatment covered a range from 34-84 % and 5-96 %, respectively, of pre-treatment activity. The slight decreases in the mean values of the activity parameters observed at the dose of 42 mg/kg bw, therefore, are not considered to be test substance-related. Habituation was not affected. All FOB and MA findings appeared to be related to the acute toxicity of imidacloprid and were completely reversible within seven days at sub-lethal doses.

Table B.6.7-2: Acute neurotoxicity study in rats – Motor and locomotor activity

FINDING	Dose level (ppm)			
	0	42	151	307
Motor activity, males, day 0				
- absolute counts	318	302	237	87
- in % of pretreatment activity	60.7	49.8	40.1	18.6
Motor activity, females, day 0				
- absolute counts	504	366	263	96
- in % of pretreatment activity	50.0	47.9	31.5	10.3
Locomotor activity, males, day 0				
- absolute counts	116	105	92	26
- in % of pretreatment activity	49.5	37.9	32.4	12.5
Locomotor activity, females, day 0				
- absolute counts	166	124	89	18
- in % of pretreatment activity	42.5	36.4	28.5	5.7

Clinical chemistry, haematology: At 151 mg/kg a decrease in serum triglycerides for males and females was found. Additional effects in animals that survived the high dose consisted of decreased serum potassium and cholesterol for females and decreased serum alanine aminotransferase (ALT) activity for males and females. Haematological findings were limited to the high-dose females and are attributed to stress and possible dehydration related to this being a lethal dose.
Gross pathology, organ weights, histopathology: Treatment-related gross lesions and effects on brain weight were not observed for males and females at any dose level. No treatment-related microscopic lesions in skeletal muscle or neural tissues were found. No evidence of a specific neurotoxic potential was seen.

Conclusion:
NOAEL overall: 42 mg/kg bw based on behavioural effects and clinical signs at 151 mg/kg bw. NOAEL neurotoxicity: 42 mg/kg bw based on behavioural effects.

B.6.7.1.2 Subchronic oral

Report: Sheets, L.P. and Hamilton, B.F. (1994b)
A subchronic dietary Neurotoxicity Screening study with Technical Grade imidacloprid (NTN 33893) in Fischer 344 Rats.
Miles Incorporation, unpublished report No.: BC7331, date: 13.6.1994

GLP: Yes (certified laboratory)

Guideline: EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617.

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 2030030, purity: 97.6 % - 98.8 %
Test animals: Fischer 344 rats (Strain CDF(F-344)/BR; Breeder Sasco, Inc., Madison, WI, USA)
Imidacloprid was administered in the diet for 13 weeks to rats (18/sex/dietary level), using analytically confirmed concentrations of 0, 140, 963 and 3027 ppm for males and females. The doses were equivalent to doses of 0, 9.3, 63.3 and 196 mg/kg b.w per day in males and to 0, 10.5, 69.3 and 213 mg/kg bw per day in females. 12 rats/sex/dietary level were used for neurobehaviour evaluation and half of them for neuropathology. Six rats/sex/dietary level were used as satellite animals for clinical pathology. The following observations and measurements were included in the study: Clinical observations, mortality, body weight, food consumption, automated measurements of activity (figure-eight maze), functional observation battery, brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were examined histopathologically.

Findings:
General observations: There were no deaths prior to terminal sacrifice, and no compound-related clinical signs were observed at any dietary level. Body weight and food consumption were reduced by treatment at doses of 963 or 3027 ppm for males and females. Ophthalmoscopic examination did not reveal compound-related ophthalmic findings.
Functional observational battery (FOB), motor and locomotor activity (MA): In the FOB, treatment-related effects (increases in the incidence of animals with slightly uncoordinated air righting response during week 13, decreased forelimb grip strength in week 8; the latter in about the same magnitude as the difference in body weight) were observed in males at the 3027 ppm dose level but not in females at any dose level. MA were not affected in males and females at any dose level.
Clinical chemistry: Decreased triglyceride levels, lactate dehydrogenase and creatine kinase activities were established for the middle and high dose group.
Gross pathology, organ weights, histopathology: No treatment-related gross lesions were observed at necropsy in males and females. Brain weight was not affected in both sexes. There were no treatment-related microscopic lesions in skeletal muscle or neural tissues.

Conclusion:
NOAEL subchronic neurotoxicity: 3027 ppm, equivalent to 196 mg/kg bw/day for males and 213 mg/kg bw/day for females (highest dose tested). NOAEL overall: 140 ppm, equivalent to 9.3 mg/kg bw/day for males and 10.5 for females based on reduced body weights and food consumption at 963 ppm.

B.6.7.1.3 Developmental neurotoxicity

Report: Sheets, L.P. (2001)
A developmental neurotoxicity screening study with technical grade imidacloprid in Wistar rats.
Bayer Corporation, unpublished report No.: 110245, date: 14.09.2001

GLP: Yes (certified laboratory)

Guideline: OPPTS 870.6300.

Deviations: The period of compound administration was extended (from pregnancy day 0 through lactation day 21, rather than from pregnancy day 6 to lactation day 10 as requested in the guideline).

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 803-0273, purity: 98.2 - 98.4 %
Test animals: female Wistar rats (Strain Crl:W(HAN)BR, Breeder Charles River Laboratories)
Imidacloprid was administered in the diet from pregnancy day 0 through lactation day 21 to groups of 30 mated female Wistar rats at nominal concentrations of 0, 100, 250 or 750 ppm. Analytically confirmed were concentrations of 0, 95.5, 227 and 691 ppm. The average daily intakes of active ingredient during different phases of the study are shown in Table B.6.8-10. Offspring were fed the control diet after weaning. On postnatal day (PND) 4, litters with a minimum of eight pups, including at least three per sex, were culled to yield as closely as possible four males and four females. Litters not meeting the selection criteria were discarded. Subsets of surviving offspring, representing at least 20 litters per level, were subjected to evaluation using the following observations and measurements: detailed clinical observations (an abbreviated functional observational battery, FOB) and developmental landmarks, body weight, food consumption, automated measurements of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance and a water maze task), and ophthalmic examination. Tissues were collected for microscopic examination on PND 11 (brain) and at study termination (brain and an assortment of other neural tissues) from selected animals (10/sex/dietary level at each age, representing a minimum of 20 litters).

Table B.6.7-3: Developmental neurotoxicity study in rats – Test substance intake

	Test substance intake (mg/kg bw/d)		
Feed concentration:	**95.5 ppm**	**227 ppm**	**691 ppm**
Females, pregnancy	8.2	19.9	56.5
Females, lactation	12.8-19.5	30.0-45.8	80-155

Findings:
Maternal: There were no compound-related clinical signs or effects on body weight, reproduction parameters and on FOB. Food consumption was lower at 691 ppm during the last week of pregnancy and the first week of lactation.

Table B.6.7-4: Developmental neurotoxicity study in rats - Maternal data

FINDING	Dose level (ppm)			
	0	**95.5**	**227**	**691**
Food consumption, pregnancy [g/kg/d]	91.6	85.8	85.8	82.1
Food consumption, lactation [g/kg/d]	173.3	172.9	172.8	175.7
Body weight gain, pregnancy [g]	104.5	109.4	107.4	101.3
Body weight gain, lactation [g]	28.3	30.6	28.8	35.1
Live litters	28/30	30/30	30/30	28/30
Litters evaluated	21	23	20	22

Offspring: The body weight gain of the high dose males and females was retarded (11-13 %) relative to controls from PND 0 through weaning on PND 21. Following the discontinuation of dosing some catch-up growth was observed for these animals until PND 60. Landmarks of sexual maturation were unaffected at any dose level.

Measures of activity in the figure-eight maze were lower at 691 ppm on PND 17 (males and females) and on PND 21 (females only). At these time points during development offspring already eat solid food in addition to suckling and thus were likely to be directly exposed to the test compound. Motor and locomotor activities were comparable to control values on PND 60 after offspring had been switched to the control diet.. There was no effect on habituation on any test occasion. With all other behavioural endpoints no evidence for treatment-induced changes was found. No morphologic changes in neural tissues were seen in the histopathological investigations.

Table B.6.7-5 Developmental neurotoxicity study in rats - Offspring data

FINDING	Dose level (ppm)			
	0	**95.5**	**227**	**691**
Mean litter size	10.6	11.3	11.0	11.5
Mean body weight at birth [g]	5.8	5.7	5.8	5.6
Mean body weight at weaning [g]	45.5	45.8	44.5	40.5++
Mean body weight PND 60, males [g]	308	321	310	297+
Mean body weight PND 60, females [g]	191	199	196	190
Motor activity PND 17, males < 200	4/15	3/16	5/16	**8/15**
Motor activity PND 17, females <200	3/16	4/16	8/16	**11/16**
Locomotor activity PND 17, males < 50	7/15	7/16	6/16	**10/15**
Locomotor activity PND 17, females <50	6/16	8/16	8/16	**10/16**
Motor activity PND 21, males < 200	2/15	2/16	2/15	2/15
Motor activity PND 21, females <200	4/16	3/16	3/14	**7/16**
Locomotor activity PND 21, males < 50	3/15	1/16	2/15	4/15
Locomotor activity PND 21, females <50	3/16	3/16	2/14	5/16
Motor activity PND 60, males < 400	3/15	0/16	3/14	2/15
Motor activity PND 60, females < 400	1/16	5/16	2/13	2/16
Locomotor activity PND 60, males < 200	3/15	0/16	2/14	2/15
Locomotor activity PND 60, females < 200	1/16	5/16	1/13	1/16

+ $p \leq 0.05$ %, ++ $p \leq 0.01$ % (Dunnett test)

Conclusion:
No permanent neurotoxic effects of imidacloprid were detected in developing rats.
NOAEL: 227 ppm (maternal and offspring) based on decreased food consumption in the dams and on retarded body weight gain of pups and decreased motor/locomotor activity at 691 ppm.

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity of metabolites

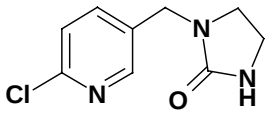
Table B.6.8-1: Summary of metabolite toxicity studies

Test system	Max. conc. / dose	Purity (%)	Results	Reference
NTN33893-urea				
Acute oral toxicity, rat	900-1480-2220-3330-5000 mg/kg bw	99.9 %	LD_{50} m / f: 4080 / 1820 mg/kg bw	Ohta, 1991a
Salmonella, E. coli/microsome test	5000 µg/plate	99.9 %	negative	Watanabe, 1991b
NTN33893-olefine				
Acute oral toxicity, rat	200-290-440-660-990-150-220-330-5000 mg/kg bw	98.0 %	LD_{50} m / f: 3500 / 1100 mg/kg bw	Ohta, 1991b
Salmonella, E. coli/microsome test	5000 µg/plate	98.0 %	negative	Ohta, 1991c
NTN33893-desnitro				
Acute oral toxicity, rat	150-240-390-630-1000 mg/kg bw	87.0 %	LD_{50} m / f: 300 / 280 mg/kg bw	Nakazato, 1991
Salmonella, E. coli/microsome test	2500 µg/plate	87.0 %	negative	Watanabe, 1991d
NTN33893-nitrosimine				
Acute oral toxicity, rat	980-1560-2500-4000 mg/kg bw	98.1 %	LD_{50} m / f: 1980 / 3560 mg/kg bw	Ohta, 1991d
Acute oral toxicity, rat	150-300-600 mg/kg bw	??	LD_{50}: > 600 mg/kg bw	Nakazato, 1988a
Acute oral toxicity, mouse	100-200-300-450 mg/kg bw	??	LD_{50} m / f: 200 / ~200 mg/kg bw	Nakazato, 1988b
Salmonella, E. coli/microsome test	5000 µg/plate	98.3 %	negative	Watanabe, 1990b
CHO-HGPRT assay	2000 µg/mL	94.3 %	negative	Lehn, 1989b
V79-HGPRT assay	2000 µg/mL	98.9 %	negative	Lehn, 1989c
Rec-assay, *B. subtilis*	2000 µg/plate	98.1 %	negative	Watanabe, 1991c
Rat hepatocyte unscheduled DNA synthesis (UDS)	1333 µg/mL	98.9 %	negative	Fautz, 1989
Chromosome aberration, CH-V79 cells	1000 µg/mL	98.8 %	negative	Heidemann, 1989
Chromosome aberration, CHO-K1 cells	1000 µg/mL	??	negative	Usami, 1988a
Mouse oral micronucleus test	0-40-80-160 mg/kg bw	96.4 %	(negative)	Usami, 1988b
Mouse oral micronucleus test	0-100 mg/kg bw	98.9 %	negative	Herbold, 1989e
Mouse i.p. micronucleus test	0-20-40-80 mg/kg bw	96.4 %	(negative)	Usami, 1988c
Mouse i.p. micronucleus test	0-50 mg/kg bw	98.9 %	negative	Herbold, 1989f
90-day oral toxicity, rat	0-100-300-1000 ppm (drinking water)	97.6-99.9 %	NOAEL: 13 mg/kg bw/d (100 ppm)	Kroetlinger, 1992

The animal and plant metabolites NTN 33893-urea, -olefine, -nitrosimine and -desnitro were tested for acute oral toxicity in rats and for the induction of point mutations in *S. typhimurium* and *E. coli*. The acute oral toxicity was found to be lower than that of the parent compound, except for NTN 33893-desnitro, and no point mutations were induced by any of the metabolites.
Because of its nitrosimine structure additional genotoxicity tests were performed with NTN 33893-nitrosimine. The following tests gave negative results: HGPRT-test (CHO and V79 cells), REC-assay, UDS-test, chromosome aberration assay (CHO cells), mouse-micronucleus test (oral and i.p. application).

In a subchronic rat drinking water study no critical findings were obtained for NTN 33893-nitrosimine. The NOAEL was 100 ppm, equivalent to 13 mg/kg bw/day.

NTN 33893-urea



Synonyma: NTN 33519, M12

Acute oral toxicity

Report:	Ohta, K. (1991) NTN 33519 – Acute oral toxicity study on rats. Nihon Bayer Agrochem K.K., unpublished report No.: RA91023, date: May 31, 1991
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	Japanese MAFF Guideline; OECD 401; FIFRA § 81-1; EEC B.1.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33519 (NTN33893-urea), batch no. TX040391, purity: 99.9 %
Test animals: Sprague Dawley rats (Strain Crj:CD (SPF); Breeder Charles River Japan)
NTN33893-urea was suspended in polyethylene glycol 400. The dosing solutions were administered by stomach tube to 5 male and 5 female fasted Sprague Dawley rats at concentrations of 5000, 3330, 2220, 1480 and 990 mg/kg bw. Application volume: 10 mL/kg bw. The observation period lasted for 14 days.

Findings:

Table B.6.8-2: NTN33893-urea – Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
1480	0	4	5	15 m – 1 h	--
990	0	3	5	10 m – 1 h	--
2220	0	5	5	15 m – 2 d	--
3330	2	5	5	10 m – 1 d	1 d
5000	3	5	5	15 m – 2 d	1 d
LD50: 4080 mg/kg bw					
Females					
990	0	3	5	30 m – 4 h	--
1480	2	2	5	3 h	1 d
2220	3	4	5	30 m – 3 h	8 h – 1 d
3330	2	5	5	25 m – 1 d	1 d
5000	2	5	5	2 h – 2 d	5 h – 1 d
LD50: 1820 mg/kg bw					

* 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Clinical signs: Mydriasis, abnormal gait, sedation, abnormal respiration, salivation, tremor.
Gross necropsy: Lung: dark reddish brown with reddish hepatisation in 2 males; trachea: retention of mucous fluid; thymus: reddish brown; kidney: congestion; intestine: yellowish contents, stomach: dark reddish brown.

Conclusion:
NTN 33893-urea is of moderate toxicity to rats following acute oral administration.

Genotoxicity testing

Report: Watanabe, M. (1991)
NTN 33519 – Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*).
Nihon Bayer Agrochem K.K., unpublished report No.: RA91024, date: July 22, 1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

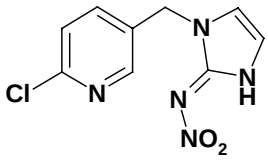
Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33519 (NTN33893-urea), batch no. TX040391, purity: 99.9 %
Test animals: *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, *E. coli* WP2uvrA
NTN33893-urea was tested in this *Salmonella-E. coli*/microsome assay at concentrations of up to and including 5000 µg/plate with and without metabolic activation. The solvent was DMSO. AF2, 2AA, NaN_3 and 9-AA were used as positive controls.

Findings:
NTN33893-urea concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.

Conclusion:
NTN 33893-urea is considered to be non-mutagenic in the salmonella/microsome test.

NTN 33893-olefine



Synonyma: NTN 35884, M6

Acute oral toxicity

Report:	Ohta, K. (1993) NTN 35884 – Acute oral toxicity study on rats. Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91039, date: 29.11.1991
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	Japanese MAFF Guideline No. 3850; OECD 401, FIFRA § 81-1; EEC B.1. Deviations: none
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: NTN 35884 (NTN 33893-olefine), batch no. TX221190, purity: 98.0 %,
Test animals: Sprague Dawley rats (Strain Crj:CD (SPF); Breeder Charles River Japan)
NTN 33893-olefine was suspended in polyethylene glycol 400. The dosing solutions were administered by stomach tube to 5 males and 5 female fasted Sprague Dawley rats at concentrations of 5000, 3300, 2200, 1500, 990, 660 and 440 mg/kg for males and 1500, 990, 660, 440, 290 and 200 mg/kg for females. Application volume: 10 mL/kg bw and 20 mL/kg bw at the dose level of 5000 mg/kg. The observation period lasted for 14 days.

Findings:
Clinical signs: Mydriasis, abnormal respiration, tremor, lacrimation, chromodacryorrhea, emaciation, abnormal gait, red urine, piloerection.

Table B.6.8-3: NTN 33893-olefine – Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
440	0	5	5	1 h – 2 d	--
660	0	5	5	2 h – 3 d	--
990	0	5	5	25 m – 3 d	--
1500	0	5	5	25 m – 4 d	--
2200	1	5	5	1 h – 6 d	4 d
3300	2	5	5	1 h – 7 d	5 d – 7 d
5000	4	5	5	1 h – 9 d	5 d – 7 d
LD50: 3500 mg/kg bw					
Females					
200	0	3	5	2 h – 4 h	--
290	0	4	5	2 h – 5 h	--
440	0	4	5	1 h – 4 h	--
660	0	3	5	1 h – 8 d	--
990	4	5	5	1 h – 6 d	3 d – 6 d
1500	4	5	5	1 h – 7 d	2 d – 4 d
LD50: 1100 mg/kg bw					

* 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Gross necropsy: spleen: reddish brown with red hepatisation, atrophy; lung: reddish brown; urinary bladder: small casts, retention of red fluid; digestive tract: dark reddish brown; stomach: mucosal thinning; intestine: yellow contents, dilated lumen and mucous dark reddish brown. No abnormal findings were observed in surviving animals.

Conclusion:
NTN 33893-olefine is of moderate toxicity to rats following acute oral administration.

Genotoxicity testing

Report: Ohta, K. (1993)
NTN 35884 – Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*).
Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91040, date: 29.11.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: None

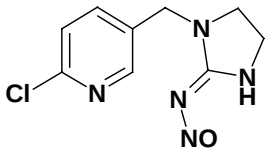
Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 35884 (NTN 33893-olefine), batch no. TX221190, purity: 98.0 %
Test animals: *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, *E. coli* WP2uvrA
NTN 33893-olefine was tested in this *Salmonella-E. coli*/microsome assay at concentrations of up to and including 5000 µg/plate with and without metabolic activation. The solvent was DMSO. AF2, 2AA, NaN_3 and 9-AA were used as positive controls.

Findings:
NTN 33893-olefine concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.

Conclusion:
NTN 33893-olefine is considered to be non-mutagenic the salmonella/microsome test.

NTN 33893-nitrosimine



Synonyma: WAK 3839, NTN 37571, M7

Acute oral toxicity

Report:	Ohta, K. (1991) WAK 3839 – Acute oral toxicity study on rats. Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91017, date: 11.3.1991
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.1 %
Test animals: Sprague Dawley rats (Strain Crj:CD (SPF); Breeder Charles River Japan)
NTN 33893-nitrosimine was suspended in polyethylene glycol 400. The dosing solutions were administered by stomach tube to 5 male and 5 female fasted Sprague Dawley rats at concentrations of 4000, 2500, 1560 and 980 mg/kg bw. Application volume: 10 mL/kg bw. The observation period lasted for 14 days.

Findings:
Clinical signs: Mydriasis, tremor, sedation, exophtalmos, abnormal respiration, emaciation, chromodacryorrhea, nasal bleeding, convulsion, abnormal gait.
Gross necropsy: The following findings were made in animals that died: lung: dark reddish brown; stomach: mucosal thinning, mucosal thickening, mucosal redness, dark reddish brown; small intestine: yellowish contents, dilated lumen, mucosal redness; spleen: atrophy; trachea: retention of foamy fluid; urinary bladder: reddish brown, retention of black brown fluid. No abnormal findings were present in surviving animals.

Table B.6.8-4: NTN 33893-nitrosimine – Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
980	0	5	5	25 m – 3 d	--
1560	3	5	5	30 m – 3 d	2 h – 3 h
2500	3	5	5	25 m – 7 d	3 d – 9 d
4000	3	5	5	1 h – 7 d	2 h – 5 d
LD50: 1980 mg/kg bw					
Females					
980	0	5	5	40 m - 1 d	--
1560	0	5	5	25 m – 2 d	--
2500	1	5	5	30 m – 6 d	5 h
4000	3	5	5	25 m – 9 d	3 h – 4 d
LD50: 3560 mg/kg bw					

∗ 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Conclusion:
NTN 33893-nitrosimine is of moderate toxicity to rats following acute oral administration.

Report: Nakazato, Y. (1990)
NTN 37571 – Oral acute toxicity study on rats.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RS89007, date: 19.1.1988

GLP: No

Guideline: In main accordance to OECD 401.

Deviations: Study report is insufficient to evaluate deviations

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: NTN 37571 (NTN 33893-nitrosimine), mixed batch no. F-4044, TX160888, purity not reported
Test animals: Sprague Dawley rats (Strain Crj:CD; Breeder Charles River Japan)
NTN 33893-nitrosimine was first dissolved in DMSO and then suspended in polyethylene glycol 400. The dosing solutions were administered to 5 males and 5 female fasted Sprague Dawley rats at concentrations of 600, 300 and 150 mg/kg bw. Non-fasted animals (2-

5/sex/doselevel) were given 900, 600, 300/350 and 150 mg/kg bw. Application volume: 10 mL/kg bw. The observation period lasted for 7 days.

Findings:
Clinical signs: Sedation.
Gross necropsy: No specific findings.

Table B.6.8-5: NTN 33893-nitrosimine – Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
150	0	2	5	1 h – 4 h	--
300	0	5	5	1 h – 1 d	--
600	0	5	5	50 m – 1 d	--
LD50: > 600 mg/kg bw					
Females					
150	0	5	5	1 h – 5 h	--
300	0	4	5	50 m – 1 d	--
600	0	5	5	1 h – 1 d	--
LD50: > 600 mg/kg bw					

* 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Conclusion:
NTN 33893-nitrosimine did not elicit mortality in rats following acute oral doses of up to 600 mg/kg bw.

Report: Nakazato, Y. (1990)
NTN 37571 – Acute toxicity study on mice.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RS88038: 19.10.1988

GLP: No

Guideline: In main accordance to OECD 401.

Deviations: Study report is insufficient to evaluate deviations

Acceptability: The study is considered to be suppplementary.

Material and Methods:
Test material: NTN 37571 (NTN 33893-nitrosimine), mixed batch no. TX160888, TX250888, TX060988, purity not reported
Test animals: ICR Mice (Strain Crj:CD-1; Breeder Charles River Japan)
NTN 33893-nitrosimine was first dissolved in DMSO and then suspended in polyethylene glycol 400. The dosing solutions were administered to 5 males and 5 female fasted ICR mice at concentrations of 450, 300, 200 and 100 mg/kg bw. Application volume: 10 mL/kg bw. The observation period lasted for 7 days.

Findings:
Clinical signs: Abnormal gait, abnormal respiration, exophthalmos, tremor, convulsion, chick-like vocalisation.
Gross necropsy: No specific findings.

Table B.6.8-6: NTN 33893-nitrosimine – Acute oral toxicity in mice

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
100	0	3	5	10 m – 2 h	--
200	3	5	5	5 m – 2 h	15 m – 20 m
300	4	5	5	3 m – 3 h	10 m – 35 m
450	4	5	5	3 m – 1 d	10 m
LD50: 200 mg/kg bw					
Females					
100	1	2	5	10 m – 2 h	30 m
200	1	5	5	5 m – 4 h	1 h
300	4	5	5	4 m – 45 m	10 m – 40 m
450	4	5	5	3 m – 1 d	7 m – 30 m
LD50: ~ 200 mg/kg bw					

* 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Discussion:
Unusual vocalisation has also been observed in the carcinogenicity study with mice at the highest dose level (420 mg/kg bw/day). Isotope dilution analysis in the urine of these mice after one year of treatment demonstrated the presence of NTN 33893-nitrosimine at a concentration of approximately 1.5 mg/100 mL of urine. It can be concluded that the metabolite is responsible for this behavioural finding.

Conclusion:
NTN 33893-nitrosimine is of moderate toxicity to mice following acute oral administration.

Genotoxicity testing

Report: Watanabe, M. (1990)
WAK 3839 – Reverse mutation assay *(Salmonella typhimurium* and *Escherichia coli).*
Nihon Tokushu Noyaku Seizo, unpublished report No.: RA90035, date: November 26, 1991

GLP: Yes (certified laboratory)

Guideline: Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295.

Deviations: none

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.3 %
Test animals: *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, *E. coli* WP2uvrA
NTN33893-nitrosimine was tested in this *Salmonella-E. coli*/microsome assay at concentrations of up to and including 5000 µg/plate with and without metabolic activation. The solvent was DMSO. AF2, 2AA, ENNG and 9-AA were used as positive controls.

Findings:
WAK 3839 concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.

Conclusion:
NTN 33893-nitrosimine is considered to be non-mutagenic in the salmonella/microsome test.

Report:	Lehn, H. (1989) WAK 3839 – Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro. Bayer AG, unpublished report No.: 17757, date: 22.2.1989
GLP:	Yes (certified laboratory). Deviations: none
Guideline:	OECD 476; 88/302/EEC; FIFRA PB 84-233295.
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 94.3 %
NTN 33893-nitrosimine was evaluated for mutagenic effects at the hypoxanthine-guanine phosphoribosyl transferase locus (forward mutation assay) in Chinese hamster ovary cells after in vitro treatment at concentrations of up to 2 mg/mL without and with S9 mix (solvent: DMSO). Ethylmethanesulfonate (without S9 mix) and dimethylbenzanthracene (with S9 mix) served as positive controls.

Findings:
Under both treatment conditions with and without exogenous metabolic activation, NTN 33893-nitrosimine induced moderate cytotoxic effects as seen by decreases in cloning efficiency in only one of the two trials each when tested up to its limit of solubility under culture conditions. There were no dose-related or reproducible increases in mutant frequency from treatment with NTN 33893-nitrosimine in comparison to the negative controls. In contrast, the positive controls showed clear mutagenic effects in the assay.

Conclusion:
NTN 33893-nitrosimine is considered to be non-mutagenic in the CHO-HPRT Forward Mutation Assay, both with and without metabolic activation.

Report: Lehn, H. (1989)
WAK 3839 – Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro.
Bayer AG, unpublished report No.: 18281, date 15.8.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 476; 88/302/EEC; FIFRA PB 84-233295.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9 %
NTN 33893-nitrosimine was evaluated for mutagenic effects at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster lung cell line V79 after in vitro treatment at concentrations of up to 2 mg/mL without and with S9 mix (solvent: DMSO). Ethylmethanesulfonate (without S9 mix) and dimethylbenzanthracene (with S9 mix) served as positive controls.

Findings:
Under non-activation conditions NTN 33893-nitrosimine induced only moderate cytotoxic effects when tested up to its limit of solubility under culture conditions. Under activation conditions strong cytotoxic effects such as decreases in relative population growth and cloning efficiency were induced. There was no significant dose-related or reproducible increase in mutant frequency in comparison to the negative controls. In contrast, the positive controls produced a clearly mutagenic effect in the assay.

Conclusion:
NTN 33893-nitrosimine is considered to be non-mutagenic in the V79-HPRT Forward Mutation Assay, both with and without metabolic activation.

Report: Watanabe, M. (1991)
WAK 3839 – Rec-assay with spores in the bacterial system.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91015, date: 1.3.1991

GLP: Yes (certified laboratory). Deviations: none

Guideline: Japanese MAFF Guideline No. 4200.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.1 %
Test animals: *Bacillus subtilis* strains H17 (rec+) and M 45 (rec-)
NTN 33893-nitrosimine was tested in the Rec-assay with *B. subtilis* for DNA-damaging effects up to and including 2000 µg/plate with and without metabolic activation. MMC and 2-AA were used as positive control substances. The solvent control was DMSO.

Findings: Growth inhibition for both strains were not observed at WAK 3839 concentrations of up to 2000 µg/plate with and without metabolic activation.

Conclusion:
NTN 33893-nitrosimine did not induce DNA damage in the Rec-assay.

Report: Fautz, R. (1989)
Unscheduled DNA synthesis in primary hepatocytes of male rats in vitro with WAK 3839.
Cytotest Cell Research GmbH & Co KG, unpublished report No.: R 4746, date: 24.4.1989

GLP: Yes (certified laboratory)

Guideline: OECD 482; EEC 88/302; EPA FIFRA (1986).

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9 %
Test animals: male Wistar rats (Strain Wistar CF HB; Breeder SAVO-Ivanovas, Kissleg, Germany)
NTN 33893-nitrosimine was tested for mutagenic effects in the in vitro rat primary hepatocyte unscheduled DNA (UDS) assay. Rat primary hepatocytes were exposed to NTN 33893-nitrosimine at concentrations from about 133.333 µg/mL to 1333.33 µg/mL. The solvent was DMSO. 2-Acetyl aminofluorene was used as the positive control.

Findings:
NTN 33893-nitrosimine did not induce reproducible significant changes in the nuclear grain counts and net grain counts of rat primary hepatocytes for the applied concentration ranges. The positive control demonstrated a good sensitivity of this assay.

Conclusion:
NTN 33893-nitrosimine is considered to be non-mutagenic in the rat primary hepatocyte UDS assay.

Report: Heidemann, A. (1989)
Chromosome aberration assay in Chinese hamster V79 cells in vitro with WAK 3839.
Cytotest Cell Research, Report No. R 4849, date: 27.9.1989

GLP: Yes

Guideline: OECD 473; EEC B.10; EPA FIFRA (1986); Japan (1984).

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.8 %
The in vitro potential of NTN 33893-nitrosimine to induce structural chromosome aberrations was tested in the chromosome aberration assay in the Chinese hamster cell line V79 at concentrations of up to 1 mg/mL without and with S9 mix (solvent: DMSO). Ethylmethanesulfonate (without S9 mix) and cyclophosphamide (with S9 mix) served as positive controls.

Findings:
Colony forming ability was reduced to 60 % after treatment with 1 mg/mL without S9-mix in the pre-test designed to study cytotoxicity; no effect on plating efficiency was seen in the presence of S9-mix. Higher concentrations could not be tested due to precipitation in the culture medium. The mitotic index was reduced at the highest concentration at fixation intervals 18 h (without S9-mix) and 7 h and 28 h (with S9-mix). There was no relevant increase in cells with structural aberrations without or with metabolic activation by S9-mix at any fixation interval. In contrast, the positive controls produced mutagenic effects in the assay.

Conclusion:
NTN 33893-nitrosimine does not induce structural chromosome aberration in the V79 Chinese hamster cell assay, both with and without metabolic activation.

Report: Usami, M. (1988a)
NTN 37571 – *In vitro* cytogenetic assay measuring chromosome aberrations in CHO-K1 cells – A Pilot Study.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RP88008, date: November 5, 1988

GLP: No

Guideline: In main accordance to OECD 473

Deviations: Pilot study; only 100 metaphases evaluated at each dose level

Acceptability: The study is considered to be not acceptable.

Material and Methods:
Test material: NTN 37571 (NTN 33893-nitrosimine), batch no. TX060988, purity not reported
NTN 33893-nitrosimine was tested in this *in vitro* cytogenetic study measuring chromosome aberration induction at concentrations of 1.0, 0.5 and 0.25 mg/mL with and without metabolic activation. Two cultures with 50 metaphases each were evaluated at each concentration. The solvent was DMSO. MNNG and DMN were used as positive controls.

Findings:
No significant increase of the frequency in chromosome aberrations was observed in the presence or absence of the metabolic activation system.

Conclusion:
NTN 33893-nitrosimine is considered to be negative in the chromosome aberration assay.

Report:	Usami, M. (1988b) NTN 37571 – Micronucleus test on the mice after oral treatment – Pilot Study. Nihon Tokushu Noyaku Seizo, unpublished report No.: RS88040, date: November 29, 1988
GLP:	No
Guideline:	In main accordance to OECD 474
Deviations:	Small study size; no individual data available
Acceptability:	The study is considered to be supplementary

Material and Methods:
Test material: NTN 37571 (NTN 33893-nitrosimine), batch no. TX19088, purity 96.4 %
Test animals: Male BDF1 mice (Breeder Charles River Japan)
NTN 33893-nitrosimine was dissolved in DMSO and diluted in PEG 400. Male BDF1 mice received the test article in a single oral gavage administration of 0, 40, 80 or 160 mg/kg bw. Mitomycin C was injected intraperitoneally as positive control at a dose of 4 mg/kg bw. The pilot study was performed to indicate a possible clastogenic potential on the chromosomes of bone marrow erythrocytes.

Findings:
No indications of a clastogenic effect in the polychromatic erythrocytes of the bone marrow were observed.

Conclusion:
The study is not of sufficient quality to determine that NTN 33893-nitrosimine is negative in the micronucleus test after oral administration.

Report: Usami, M. (1988c)
NTN 37571 – Micronucleus test on the mice after i.p. treatment – Pilot Study.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RS88041, date: November 29, 1988

GLP: No

Guideline: In main accordance to OECD 474

Deviations: Small study size; no individual data available

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: NTN 37571 (NTN 33893-nitrosimine), batch no. TX19088, purity 96.4 %
Test animals: male BDF1 mice (Breeder Charles River Japan)
NTN 33893-nitrosimine was dissolved in DMSO and diluted in olive oil. Male BDF1 mice received the test article in a single intraperitoneal administration of 0, 20, 40 or 80 mg/kg bw. Mitomycin C was injected intraperitoneally as positive control at a dose of 4 mg/kg bw. The pilot study was performed to indicate a possible clastogenic potential on the chromosomes of bone marrow erythrocytes.

Findings:
No indications of a clastogenic effect in the polychromatic erythrocytes of the bone marrow were observed.

Conclusion:
The study is not of sufficient quality to determine that NTN 33893-nitrosimine is negative in the micronucleus test after intraperitoneal administration.

Report: Herbold, B. (1989e)
WAK 3839 – Micronucleus test on the mouse after oral application.
Bayer AG, unpublished report No.: 18406, date: 3.10.1989

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 474; EEC B.1; US EPA 1984 (PB 84-233295

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9 %
Test animals: NMRI mice (Strain Bor:NMRI (SPF Han); Breeder Winkelmann, Borchen, Germany

NTN 33893-nitrosimine was tested for clastogenic effects using the micronucleus test on the mouse in vivo following a single oral administration of 100 mg/kg bw. NTN 33893-nitrosimine was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and administered at a dose of 20 mg/kg bw. Administration volume was 10 mL/kg bw. Smears were prepared from femoral bone marrow at 24, 48 and 72 hours postdose.

Findings:
Animals treated with NTN 33893-nitrosimine showed symptoms of toxicity for up to two hours after administration. All animals survived until the end of the study. The ratio of polychromatic to normochromatic erythrocytes was not altered. No indications of a clastogenic effect were found. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
NTN 33893-nitrosimine is considered to be negative in the micronucleus test after oral administration.

Report:	Herbold, B. (1989f) WAK 3839 or NTN 37571 – Micronucleus test on the mouse after intraperitoneal application. Bayer AG, unpublished report No.: 18407, date 3.10.1989
GLP:	Yes (certified laboratory)
Guideline:	OECD 474; EEC B.12; US EPA 1984 (PB 84-233295).
Deviations:	None
Acceptability:	The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9 %
Test animals: NMRI mice (Strain Bor:NMRI (SPF Han); Breeder Winkelmann, Borchen, Germany
NTN 33893-nitrosimine was tested for clastogenic effects using the micronucleus test on the mouse in vivo following a single intraperitoneal administration of 50 mg/kg bw. NTN 33893-nitrosimine was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and administered at a dose of 20 mg/kg bw. The administration volume was 10 mL/kg bw. Smears were prepared from femoral bone marrow at 24, 48 and 72 hours postdose.

Findings:
Animals treated with NTN 33893-nitrosimine showed symptoms of toxicity for up to two hours after administration. All animals survived until the end of the study. The ratio of polychromatic to normochromatic erythrocytes was not altered. No indications of a clastogenic effect were found. The results for the positive control indicated a clear clastogenic effect.

Conclusion:
NTN 33893-nitrosimine is considered to be negative in the mouse micronucleus test after intraperitoneal application.

Subchronic oral toxicity

Report: Kroetlinger, F. (1992)
WAK 3839 – Subchronic toxicological study on rats (twelve-week administration in drinking water).
Bayer AG, unpublished report No.: 21140, date: 2.3.1992

GLP: Yes (certified laboratory).

Guideline: OECD 408;

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: WAK 3839 (NTN 33893-nitrosimine), various batches, purity 97.6 - 99.9 %
Test animals: Wistar rats (Strain Bor:WISW (SPF-Cpb); Breeder Winkelmann, Borchen, Germany)
NTN 33893-nitrosimine was administered to groups of 15 males and 15 females rats of the strain Bor:WISW (SPF-Cpb) in an unlimited supply of drinking water over a period of 12 weeks at dose levels of 0, 100, 300 and 1000 ppm. The 1000 ppm level represented a concentration near the saturation point. Mean consumption of WAK 3839 per kg body weight and day were: 13, 35 and 106 mg for males and 13, 39 and 117 mg for females

Findings:
General findings: Appearance, behaviour and mortality of the animals gave no evidence for a treatment-related effect at levels up to 1000 ppm. Also food intake and body weight development were not affected to a toxicologically significant extent during the entire study. The water intakes were decreased by up to 16 % in the 1000 ppm dose groups.
Haematology, clinical chemistry, urinalysis: There were no relevant treatment-related effects on red blood cell parameters up to and including 1000 ppm. At 300 ppm and 1000 ppm increased lymphocytes counts and lower numbers of polymorphonuclear cells were observed.

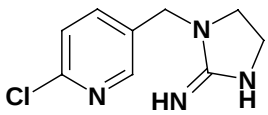
Table B.6.8-7: NTN 33893-nitrosimine - Subchronic oral toxicity in rats - Haematology

Dose	0 ppm	100 ppm	300 ppm	1000 ppm
Males				
Leuco [10E9/L]	5.5	7.7	7.2	6.5
Lym [%]	88.8	91.1	92.7	94.2
Segm [%]	9.1	6.9	4.7	4.5
Females				
Leuco [10E9/L]	5.2	5.0	6.2	4.8
Lym [%]	90.0	90.5	93.2	94.0
Segm [%]	7.8	6.9	5.4	4.8

Reduced sodium levels in males and females at 1000 ppm on day 29 and in females on day 85 are considered a treatment-related effect on the sodium balance. Ophthalmic examinations showed no evidence for oculotoxic effects at 1000 ppm. No signs of damage to liver or thyroid were observed at 1000 ppm. The urinalyses and examinations of urinary sediment showed no treatment-related up to and including 1000 ppm. None of the tests produced evidence for an effect on or damage to the kidneys.
Gross necropsy, organ weights, histopathology: There were no treatment-related findings.

Conclusion:
NOAEL: 100 ppm concentration, equivalent to 13 mg/kg bw/day in drinking water based on changed haematological parameters at 300 ppm.

NTN 33893-desnitro



Synonyma: NTN 38014, M9

Acute oral toxicity

Report: Nakazato, Y. (1993)
NTN 38014 – Acute oral toxicity study on rats.
Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91018, date: March 18, 1991

GLP: Yes (certified laboratory).

Guideline: Japanese MAFF Guideline No. 3850; OECD 401; FIFRA §81-1; EEC B.1

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 38014 (NTN 33823-desnitro), batch no. TX281190, purity: 87.0 %
Test animals: Sprague Dawley rats (Strain Crj:CD (SPF); Breeder Charles River Japan)
NTN 33823-desnitro was suspended in polyethylene glycol 400. The dosing solutions were administered by stomach tube to 5 males and 5 female fasted Sprague Dawley rats at dose levels of 1000, 630, 390, 240 and 150 mg/kg bw. Application volume: 10 mL/kg bw. The observation period lasted for 14 days.

Findings:

Table B.6.8-8: NTN 33823-desnitro - Acute oral toxicity in rats

Dose [mg/kg bw]	Toxicological results*			Duration of signs	Time of death
Males					
150	0	5	5	25 m – 1 d	--
240	2	5	5	20 m – 2 d	2 h – 1 d
390	4	5	5	20 m – 8 d	2 h – 3 h
630	4	5	5	15 m – 14 d	40 m – 3 h
1000	5	5	5	1 m – 50 m	2 m – 50 m
LD50: 300 mg/kg bw					
Females					
150	0	5	5	30 m – 1 d	--
240	2	5	5	25 m – 4 d	3 h – 1 d
390	4	5	5	25 m – 10 d	2 h – 3 h
630	5	5	5	15 m – 3 h	25 m – 3 h
1000	5	5	5	1 m – 40 m	6 m – 40 m
LD50: 280 mg/kg bw					

* 1st figure = number of dead animals, 2nd figure = number of animals with signs, 3rd figure = number of animals in the group

Clinical signs: Sedation, ptosis, abnormal respiration, abnormal gait, tremor, hypothermia of the skin, convulsion and red tear.
Gross necropsy: Red/brown or gray/whitish patches in lungs; reddening of mucosa in the gastrointestinal tract.

Conclusion:
NTN 33893-desnitro is of moderate toxicity to rats following acute oral administration.

Genotoxicity testing

Report: Watanabe, M. (1993)
NTN 38014 – Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*).
Nihon Tokushu Noyaku Seizo, unpublished report No.: RA91019, date: March 29, 1991

GLP: No

Guideline: Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 38014 (NTN 33823-desnitro), batch no. TX281190, purity: 87.0 %
Test systems: *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, *E. coli* WP2uvrA
NTN 33823-desnitro was tested in this *Salmonella* - *E. coli*/microsome assay at concentrations of up to and including 1250 µg/plate with and up to and including 2500 µg/plate without metabolic activation. The solvent was DMSO. AF2, 2AA, NaN_3 and 9-AA were used as positive controls.

Findings:
NTN 33823-desnitro concentrations of up to 2500 µg/plate did not produce an increase in the mutant count. Bacterial growth was inhibited at the highest doses with and without metabolic activation.

Conclusion:
NTN 33893-desnitro is considered to be non-mutagenic in this assay with and without metabolic activation.

B.6.8.2 Supplementary studies on the active substance

B.6.8.2.1 Studies on combination toxicity

Summary of combination toxicity
No synergistic or superadditive acute toxicity was seen in rats following simultaneous oral administration of imidacloprid with other insecticidal active ingredients such as cyfluthrin, methamidophos and flumethrin.

Report: Kroetlinger, F. (1994a)
NTN 33893 (c.n. imidacloprid (proposed)) FCR 1272 (c.n. cyfluthrin) – Study for combination toxicity in rats.
Bayer AG, unpublished report No.: 23420, date: 19.10.1994
+ Addendum report no. 23420A, date: 02.02.1995.

GLP: Yes (certified laboratory). Deviations: Homogeneity and concentration of dosage solutions not confirmed

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid technical, batch no. 816255037, purity 97.6 %;
Cyfluthrin, batch no. 238005176, purity 95.1 %
Test animals: Male Wistar rats (Strain HSD/WIN:WU; Breeder Harlan-Winkelmann, Borchen Germany)
Material and methods: Imidacloprid and cyfluthrin were formulated in deionised water using 2 % v/v Cremophor EL. Male rats received oral doses of either the single compounds or a combination of imidacloprid and cyfluthrin at equitoxic doses (percentages corresponding to the ratio of their LD_{50} values). The theoretically expected LD_{50} of the combination was then compared to the LD_{50} value obtained experimentally. The treatment volume was 10 mL/kg bw. The recovery period was 14 days.

Findings:
Clinical signs:
Imidacloprid: At 500 mg/kg bw, the male rats exhibited apathy, labored and/or accelerated breathing, decreased motility, staggering gait, spasmodic state, narrowed palpebral fissures, transient tremor of the head, and increased blood supply to hairless parts of the body. In addition, red-encrusted nasal wings and red nasal discharge were observed in an isolated case. Transient convulsions and tonical cramps as well as isolated cases of dyspnea and lateral position were additionally observed at higher doses. Piloerection and transient tremor occurred in one animal, and poor reflexes in another.

Cyfluthrin: At ≥ 14 mg/kg bw, the male rats exhibited apathy, labored breathing, decreased motility, uncoordinated movements, broad gait, digging activities, salivation and lacrimation, red secretion at the orbital margins, transient rolling over and shaking, and transient vocalisation. Isolated cases of piloerection and narrowed palpebral fissures were also observed. Lateral position could be observed in one animal, and transient clonic cramps in another.

Table B.6.8-9: Acute oral toxicity of imidacloprid

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
500	1/5/5	28 min – 2 d	1 d	20
600	4/5/5	30 min – 3 d	2 h 30 min – 1 d	80
750	5/5/5	27 min – 2 d	4 h 45 min – 2 d	100
LD_{50} rat: ~ 547 mg/kg bw				

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Table B.6.8-10: Acute oral toxicity of cyfluthrin

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
14	2/5/5	34 min – 2 d	1 h 45 min – 2 h	40
20	4/5/5	34 min – 2 d	1 h 30 min – 2 h	80
LD_{50} rat: ~ 15 mg/kg bw				

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Table B.6.8-11: Acute oral toxicity of the imidacloprid / cyfluthrin combination (97.33 % / 2.67 %)

Dose mg/kg bw	Toxicological result*	LD_{50} experimental [mg/kg bw]	LD_{50} expected [mg/kg bw]	Factor
315 450	1/5/5 3/5/5	414	281	0.7

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Imidacloprid and cyfluthrin: At 315 mg/kg bw, the male rats exhibited apathy, labored and/or accelerated breathing, decreased motility, staggering gait, spasmodic state, salivation, digging activities, narrowed palpebral fissures, increased blood supply to hairless parts of the body, transient shaking, tremor of the head and transient grooming activities. Piloerection, uncoordinated movements and isolated cases of broad gait were additionally observed at the higher dose. A red-encrusted muzzle could be observed in one animal, and transient rolling over in another.
Body weights: The treatment with cyfluthrin and the combination of imidacloprid and cyfluthrin exhibited no effect on the body weights throughout the recovery period. One animal treated with imidacloprid showed retarded body weight development at 600 mg/kg bw.

Conclusion:
No synergistic or superadditive acute toxicity was seen in rats following simultaneous oral administration of imidacloprid and cyfluthrin.

Report: Kroetlinger, F. (1994b)
NTN 33893 (c.n. imidacloprid (proposed)) SRA 5172 (c.n. methamidophos) – Study for combination toxicity in rats.
Bayer AG, unpublished report No.: 23454, date: 7.11.1994

GLP: Yes (certified laboratory). Deviations: Homogeneity and concentration of dosage solutions not confirmed

Guideline: OECD 401, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid technical, batch no. 816255037, purity 97.6 %,
Methamidophos, batch no. 278167052, purity 73.8 %
Test animals: Male Wistar rats (Strain HSD/WIN:WU; Breeder Harlan-Winkelmann, Borchen Germany)
Imidacloprid and methamidophos were formulated in deionised water using 2 % v/v Cremophor EL. The male rats received oral doses of either the single compounds or a combination of imidacloprid and methamidophos at equitoxic doses (percentages corresponding to the ratio of their LD_{50} values). The theoretically expected LD_{50} of the

combination was then compared to the LD_{50} value obtained experimentally. The treatment volume was 10 mL/kg bw. The recovery period was 14 days.

Findings:
Clinical signs:
Imidacloprid: At 500 mg/kg bw, the male rats exhibited apathy, labored and/or accelerated breathing, decreased motility, staggering gait, spasmodic state, narrowed palpebral fissures, transient tremor of the head, and increased blood supply to hairless parts of the body. In addition, red-encrusted nasal wings and red nasal discharge were observed in an isolated case. Transient convulsions and tonical cramps as well as isolated cases of dyspnea and lateral position were additionally observed at higher doses. Piloerection and transient tremor occurred in one animal, and poor reflexes in another.

Table B.6.8-12: Acute oral toxicity of imidacloprid

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
500	1/5/5	28 min – 2 d	1 d	20
600	4/5/5	30 min – 3 d	2 h 30 min – 1 d	80
750	5/5/5	27 min – 2 d	4 h 45 min – 2 d	100
LD_{50} rat: ~ 547 mg/kg bw				

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Table B.6.8-13: Acute oral toxicity of methamidophos

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
10	0/5/5	12 min – 1 d	--	0
13	2/5/5	7 min – 2 d	36 min	40
16	5/5/5	10 min – 45 min	29 min – 45 min	100
18	3/5/5	8 min – 3 d	31 min – 40 min	60
25	5/5/5	8 min – 1 h	21 min – 1 h	100
LD_{50} rat: ~ 15 mg/kg bw				

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Methamidophos: At 10 mg/kg bw, apathy, piloerection, labored breathing, decreased motility, palmospasms, salivation and lacrimation, dacryohemorrhea, red-encrustated muzzle and orbital margins, and transient tremor of the head could be observed in the male rats. Only in one animal was red salivation seen in addition. The animals exhibited dyspnea at higher doses. Exophthalmos and red-encrusted front paws were observed in one animal, and red secretion at the orbital margins in one another.

Table B.6.8-14: Acute oral toxicity of the imidacloprid /methamidophos combination (97.33 % / 2.67 %)

Dose [mg/kg bw]	Toxicological results*	LD_{50} experimental [mg/kg bw]	LD_{50} expected [mg/kg bw]	Factor
315 355 450	1/5/5 2/5/5 5/5/5	373	281	0.8

*1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Imidacloprid and methamidophos: At 315 mg/kg bw, the male rats exhibited apathy, piloerection, labored breathing, decreased motility, palmospasms, spasmodic state, salivation, and lacrimation, narrowed palpebral fissures, lateral position, transient tremor and tremor of the head. Absent or poor reflexes and red secretion at the orbital margins, as well as red-encrusted orbital margins and muzzle could be observed in isolated cases. At higher doses, the findings also included transient convulsions, isolated cases of dyspnea and red salivation.

Body weights: Retarded body weight development over the recovery period could be observed in one animal at 600 mg imidacloprid/kg bw, and in one at 18 mg methamidophos/kg bw. No effects on the body weights were noted in animals treated with the combination of the two test substances.

Conclusion:
No synergistic or superadditive acute toxicity was seen in rats following simultaneous oral administration of imidacloprid and methamidophos.

Report: Andrews, P. (2002)
Flumethrin & Imidacloprid (c.n. Flumethrin & Imidacloprid) – Study for acute oral combination toxicity in rats.
Bayer AG, unpublished report No.: PH 31644, date: 7.1.2002

GLP: Yes (certified laboratory). Deviations: none

Guideline: OECD 423, FIFRA § 81-1, EEC B.1.

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: Imidacloprid, batch no. 0026 T, purity 98.4 %
Flumethrin, batch no. 816458003, purity 95.8 %
Test animals: Female Wistar rats (Strain Hsd Cpb:WU; Breeder Harlan-Winkelmann, Borchen, Germany)
The purpose of this investigation was to assess possible interactions between imidacloprid and flumethrin. The test substances were formulated in deionised water using 2 % v/v Cremophor EL. Single oral doses of the test substances and one dose of their combination at equitoxic doses were administered by stomach tube to groups of 3-5 female Wistar rats. The

dose levels were selected to produce minimal clinical signs of toxicity. The treatment volume was 10 mL/kg bw. The recovery period was 14 days.

Findings:
Clinical signs:
Imidacloprid: At 150 mg/kg bw, spastic and uncoordinated gait and temporary increased motility were noted 40 min after administration up to 1 day after administration.
Flumethrin: At 5 mg/kg bw, piloerection, digging and cleaning movements, decreased reactivity and motility, uncoordinated gait, increased salivation and labored breathing were noted 1 h after administration up to 5 h after administration.
Imidacloprid and flumethrin: After simultaneous administration, piloerection, temporary digging movements, decreased reactivity and motility, uncoordinated gait, increased salivation and labored breathing were noted 2 h after administration up to 5 h after administration.
Body weights: No effects on body weights and body weight gains were observed.

Table B.6.8-15: Acute oral toxicitiy of imidacloprid

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
150	0/3/3	2 h – 2 d	--	0
200	0/3/3	40 min – 2 d	--	0
250	0/3/3	10 min – 5 h	--	0
400	1/3/3	1 h – 5 d	2 d	33

∗1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Table B.6.8-16: Acute oral toxicity of flumethrin

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
2	0/3/3	1 h – 5 h	--	0
3	0/3/3	1 h – 2 d	--	0
5	0/3/3	1 h – 5 h	--	0
10	0/3/3	50 min – 4 h	--	0

∗1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Table B.6.8-17: Acute oral toxicity of imidacloprid / flumethrin combination (96.77 % / 3.23 %)

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
females				
150 imidacloprid +5 flumethrin	0/5/5	2 h – 5 h	--	0

∗1st figure = number of dead animals; 2nd figure = number of animals with signs; 3rd figure = number of animals in the group

Conclusion:
No synergistic or superadditive acute toxicity was seen in rats following simultaneous oral administration of imidacloprid and flumethrin.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Report on medical surveillance on manufacturing plant personnel

Based on data established in periodical occupational examinations performed by the Bayer medical department, no adverse health effects have been reported for employees handling imidacloprid during the production of active ingredient and formulations (Faul and Krauthausen, 1996; Faul and Neukaeter, 1996).

B.6.9.2 Report on clinical cases and poisoning incidents

Mild cases of contact dermatitis in pet owners have been reported following use of veterinary formulations of imidacloprid [redacted] The effects can be attributed to formulation-specific component(s) of this product but not to imidacloprid itself.
After ingestion of four Lizetan combi rodlets (50 mg imidacloprid per rodlet) by a four year old child no signs of poisoning or adverse health effects have been reported. The ingested dose corresponds to ca. 10 mg imidacloprid per kg bodyweight (Steffens, 2000) which is seven to eightfold lower than the doses that elicited signs of acute toxicity in dogs.
From the experimental biological testing and from the field tests with imidacloprid formulations no negative effects on the health of the operators or workers were reported.

B.6.9.3 Observations on exposure of the general population and epidemiological studies

No epidemiological studies on the general population are available for imidacloprid.

B.6.9.4 Clinical signs and symptoms of poisoning and details of clinical tests

Information on symptoms of poisoning or clinical signs in humans are not available.

The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning.

B.6.9.5 Proposed treatment: first aid measures, antidotes, medical treatment

A specific antidote is not known. In case of an oral uptake, first aid measures should consist of removal of ingested compound by gastric lavage or induction of vomiting and symptomatic treatment. Contaminated skin should be washed immediately with plenty of water.
Administration of activated charcoal was found to be effective under experimental conditions in laboratory rodents (Watanabe, 1995).

B.6.9.6 Expected effects of poisoning

No experiences on symptoms and effects of poisoning are available for humans. In laboratory animals symptoms such as apathetic state, depressed muscular tone, respiratory disturbance, trembling, muscular cramps and bradycardia occurred at high doses.
Based on the fact that imidacloprid binds to the nicotinergic acetylcholine receptor, albeit as a weak agonist in mammals, nicotine-like action may occur also in severe intoxicated humans. Complete recovery is expected within a few days.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary

Toxicokinetics and metabolism
Following oral administration the insecticide active ingredient imidacloprid is rapidly and almost completely absorbed from the intestinal lumen of rats. Peak plasma concentrations are reached within approximately 1 - 2 hours after oral administration. The radioactivity was rapidly distributed from the intravascular space to the peripheral tissues and organs. Blood/plasma kinetics revealed initial half-lives of about 3 hrs; terminal half-lives vary between 26 and 118 hrs. The elimination from the organism is fast and complete, there is no indication of any bioaccumulation potential of the parent compound and/or its metabolites. On average, 75 % of the administered radioactivity is excreted with the urine, the remainder is found in the faeces. Most of the faecal radioactivity originates from biliary excretion. There is some evidence for enterohepatic circulation.

At the end of the test period of 48 h less than 1 % of the radioactivity remained in the tissues. Levels above average were only observed in the contents of the gastrointestinal tract, in liver, kidneys, adrenals, thyroid, connective tissues and the vascular walls of the aorta. The extent of penetration of the blood-brain barrier is very limited.

The metabolisation rate of imidacloprid in the rat is high, and somewhat more pronounced in male than in female animals. The amount of unchanged parent compound in excreta varied between 10 and 17 % of the given dose. Metabolism proceeds on two major routes, one beginning with oxidative cleavage of the methylen-bridge, the other with the hydroxylation of the imidazolidine ring in the 4-or 5-position. The main metabolites in urine are 6-chloronicotinic acid (M14) and its glycine conjugate (M15) as well as the two corresponding biotransformation products M26 and M27 which contain the imidazolidine ring. Further products detected in urine were the two monohydroxylated metabolites M1 and M2 and the unsaturated compound M6. The latter is also excreted with the faeces, together with M14 and M15.

Studies on the biokinetic and metabolic behaviour of imidacloprid and its nitrosimino plant metabolite WAK 3839 (NTN 33893-nitrosimine, M7) in male rats yielded comparable data for absorption, distribution and elimination. M7 was eliminated somewhat more rapidly, and the radioactivity levels in the organs were lower than after administration of imidacloprid. M7 was not detected in the urine or faeces following administration of single oral doses of 1 mg/kg bw or 150 mg/kg bw imidacloprid to male rats. However, after prolonged treatment

(one year) at high doses of imidacloprid in the diet, M7 was found in the urine of rats and mice at levels of 9 mg/100 mL (rat) and 1.5 mg/100 mL (mouse). Formation of M7 from imidacloprid seems to occur when enzyme systems involved in the usual degradation reactions are saturated as it is likely to be the case after chronic feeding of high imidacloprid concentrations. The formation of M7 (WAK 3839) in rats and mice has been confirmed and its toxicological properties play a role in the chronic toxicity studies with these animal species.

Acute toxicity

Imidacloprid exhibits moderate acute oral toxicity to rats. The symptoms observed following oral intake consisted of apathy, respiratory difficulties, staggering gait, decreased motility, narrowed palpebral fissures, piloerection, transient trembling and spasms, and were reversible within six days. Symptoms are considered related to the activation of α-bungarotoxin-sensitive nicotinic acetylcholine receptors. Imidacloprid exhibited high acute toxicity to rats after intraperitoneal administration; the symptoms were similar to those observed after oral exposure. The compound was found to exhibit no acute dermal toxicity and only low acute inhalative toxicity after exposure to dust. No exact assessment of the LC_{50} for acute aerosol exposure was possible, since rats tolerated inhalation of the maximum technically feasible concentration (69 mg/m^3 air) without symptoms. Imidacloprid had no irritant properties on the skin or eyes of rabbits. No skin sensitisation was observed in the maximisation test on guinea pigs.

Short term toxicity

Reduced body weight gain was the most sensitive parameter in rats, mice and dogs following short term administration of imidacloprid. Transient trembling and tremor was observed in dogs in the 28-day and the 90-day study from the first week of treatment. No tremors were reported in the one-year study which used dose levels similar to the mean exposure in the 90-day study. The NOAEL for this endpoint from all available dog studies is considered to be 40 mg/kg bw.

The liver has been found to be the main target organ after repeated administration of imidacloprid to rats, mice and dogs. The spectrum of changes observed ranged from induction of hepatic microsomal enzymes and disturbances of hepatic function to histopathologically apparent organ damage. Initial signs of an effect on the liver were induction of hepatic microsomal enzymes (elevated activities of the mixed-function oxidases, particularly cytochrome P-450) and slight hepatocellular hypertrophy and cytoplasmic lesions; increased liver weights were occasionally encountered. Higher doses affected hepatic function with dysregulation of the lipid and protein metabolism, as manifested by changes in blood levels of cholesterol, triglyceride, protein and albumin. The occasional increases in blood coagulation time and elevated total serum bilirubin levels are also attributed to disturbance of the hepatic function. In addition, signs of a direct cytotoxic effect were observed after administration of high doses. These include elevated activities of the enzymes alatin aminotransferase, alcaline phosphatase and glutanate dehydrogase in the blood as well as histopathological findings (swollen cellular nuclei, round-cell infiltration and hepatic cell necroses).

Body weight gain was a sensitive parameter in most of the short- and long-term feeding studies in rats, mice and dogs. In some studies the reduction of body weights was associated with a simultaneous increase in the feed intake, indicating poor food utilisation. This may be a possible consequence of the disturbances in lipid and protein metabolism described above.

Minimal follicular atrophy of the thyroid and some instances of a slightly depressed triiodothyronine level were found at the high dose (5000 ppm) in the 28-day oral dog study. However, no similar findings were observed in the 90-day and one-year feeding studies on dogs at doses up to and including 2500 ppm.

No evidence of treatment-related haemotoxicity or damage to the haematogenic organs was found in any study involving repeated administration of imidacloprid.

Table B.6.10-1: NOAELs and LOAELs obtained in short-term toxicity studies

Study **Dose levels** **Imidacloprid purity**	**NOAEL** **[mg/kg bw/d]**	**LOAEL** **[mg/kg bw/d]**
Oral studies		
Rat 90-day range-finding 0-120-600-3000 ppm purity: 92.8 %	males: 11 females: 15 (120 ppm)	males: 57 females: 78 (600 ppm)
Rat 90-day 0-150-600-2400 ppm purity: 95.3 %	males: 14 females: 83 (150 / 600 ppm)	males: 61 females: 422 (600 / 2400 ppm)
Mouse 90-day 0-120-600-3000 ppm purity: 92.8 %	males: 391 females: 446 (600 ppm)	males: 2408 females: 3087 (1000 ppm)
Dog 28-day 0-200-1000-5000 ppm purity: 92.0 %	7.3 (200 ppm)	31 (1000 ppm)
Dog 90-day 0-200-600-1800/1200 ppm purity: 95.3 %	23.5 (600 ppm)	45.4 (mean) but more likely 70-80 (1800/1200 ppm)
Dog 12-month 0-200-500-1250/2500 ppm purity: 94.9 %	15 (500 ppm)	42/70 (1250 /2500 ppm)
Inhalation studies		
Rat 5-day 0-20-109-505 mg/m³ air purity: 95.3 %	20 mg/m³ air	109 mg/m³ air
Rat 28-day 0-5.5-30.5-191.2 mg/m³ air purity: 95.2 %	5.5 mg/m³ air	30.5 mg/m³ air
Dermal studies		
Rabbit 15-day 0–1000 mg/kg bw/d purity: 95.0 %	1000	–

Genotoxicity

In vitro tests for point-mutation effects (Salmonella/microsome reverse-mutation and CHO-HGPRT tests) gave negative results for imidacloprid. *In vitro* tests for DNA-damaging properties (yeast mitotic recombination assay, *B. subtilis* rec-assay, rat hepatocyte UDS test) were also negative. A weak indication of sister chromatid exchange (SCE) induction in CHO cells was found in an *in vitro* test. However, this was not confirmed by the *in vivo* SCE test in bone marrow of Chinese hamsters. In the cytogenetic study with human lymphocyte cultures,

a slight, reproducible increase in the aberration rate was observed in the cytotoxic concentration range without metabolic activation; an equivocal result was obtained with metabolic activation. All *in vivo* tests for chromosome damage (micronucleus test, bone marrow cytogenetics and spermatogonia cytogenetics) were negative, however, so that a clastogenic potential of imidacloprid *in vivo* can be excluded. The overall conclusion is that imidacloprid exhibits no genotoxic or mutagenic potential *in vivo.*

Long-term toxicity and carcinogenicity

An increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in the chronic feeding study on rats. The increased incidence of this involution of isolated thyroid follicles, is considered a treatment-related premature ageing of the thyroid. An effect on thyroid function can be excluded, since the plasma levels of thyreotropin, triiodothyronine and thyroxine remained unchanged.

An increased incidence of mineralisation processes in the thalamus was seen in mice at very high dose levels. Mice in this dose group displayed behavioural abnormalities (increased vocalisation) and an apparent increase in mortality after ether narcosis for blood withdrawal. Vocalisation was also observed in an acute toxicity study in mice with M7 (NTN33893-nitrosimine) and may be related to the formation of this metabolite under prolonged high dose exposure conditions.

No evidence of an oncogenic potential of imidacloprid was found in either the rat or the mouse long-term feeding study.

Table B.6.10-2: NOAELs and LOAELs obtained in long-term toxicity studies

Study **Dose levels** **Purity**	**NOAEL** **males / females** **[mg/kg bw/d]**	**LOAEL** **males / females** **[mg/kg bw/d]**
Rat 24-month oral diet (combined chronic toxicity and carcinogenicity) 0-100-300-900-1800 ppm purity: 94.4 – 95.3 %	6 / 25 (100 / 300 ppm)	17 / 73 (300 / 900 ppm)
Mouse 24-month oral diet (carcinogenicity) 0-100-330-1000-2000 ppm purity: 94.6 – 95.3 %	66 / 104 (330 ppm)	208 / 274 (1000 ppm)

Reproductive toxicity

The reproductive toxicity of imidacloprid was investigated in a two-generation study in rats, in developmental toxicity studies in rats and rabbits and in a developmental neurotoxicity study in rats. Reproduction was not adversely affected by treatment with imidacloprid.

Table B.6.10-3: NOAELs and LOAELs obtained in reproductive toxicity studies

Study dose levels purity	Target	NOAEL [mg/kg bw/d]	LOAEL [mg/kg bw/d]
Rat 2-generation study 0-100-250-700 ppm purity: 94.4 – 95.3 %	Parental toxicity	~20 (250 ppm)	~50 (700 ppm)
	Reproduction toxicity	~50 (700 ppm)	–
	Offspring toxicity	~40 (250 ppm)	94 (700 ppm)
Rat embryotoxicity 0-10-30-100 mg/kg purity: 94.2 %	Maternal toxicity	30	100
	Developmental toxicity	30	100
Rabbit embryotoxicity 0-8-24-72 mg/kg bw/day purity: 94.2 %	Maternal toxicity	8	24
	Developmental toxicity	24	72

Reduced body weight gain was the most sensitive parameter in parents and pups. A slightly increased incidence of wavy ribs was the only prenatal effect established in rats at a dose that was toxic to the mothers. In rabbits, the body weights of the foetuses were slightly depressed in the maternally toxic dose range only. As a consequence of this growth retardation, the foetuses also showed an increased incidence of retarded ossification. Overall the data show that imidacloprid has no primary reproductive toxicity and exerts no teratogenic potential.

Neurotoxicity

In acute, subchronic and developmental neurotoxicity screening studies in rats investigating specific neurotoxicological parameters by functional observation battery, automated motor activity measurements and special neurohistopathology, behavioural changes in the acute experiment and to a much lesser extent in the developmental study were the only signs that could be indicative of neurotoxic effects. Most clinical signs appeared related to acute receptor-mediated cholinergic toxicity of this chloronicotinyl compound.

Table B.6.10-4: NOAELs and LOAELs obtained in neurotoxicity studies

Study dose levels purity	Target	NOAEL [mg/kg bw/d]	LOAEL [mg/kg bw/d]
Rat acute oral neurotoxicity 0–20–42–151-307 mg/kg bw purity: 97.6 – 98.8 %	Neurotoxicity	42	151
	General toxicity	42	151
Rat 90-day oral neurotoxicity 0–140–963–3027 ppm purity: 97.6 %	Neurotoxicity	males 196 females 213 (3027 ppm)	–
	General toxicity	males 9.3 females 10.5 (140 ppm)	males 63 females 69 (963 ppm)
Rat developmental neurotoxicity 0-95.5-227-691 ppm purity: 98.2 - 98.4 %	Maternal toxicity	56 (691 ppm)	–
	General toxicity in offspring	~30 (227 ppm)	~80 (691 ppm)
	Neurotoxicity in offspring	~80 (691 ppm)	–

Further toxicological studies

Following specific Japanese testing requirements, the animal and plant metabolites NTN 33893-urea, -olefine, -introsimine and –desnitro were tested for acute oral toxicity in rats and for the induction of point mutations in *S. typhimurium* and *E. coli*. The acute oral toxicity of the metabolites was found to be lower than that of the parent compound, except for NTN 33893-desnitro, and no point mutations were induced by any of the metabolites.

Because of a structural alert additional genotoxicity tests were performed with NTN 33893-nitrosimine. The following tests gave negative results: HGPRT-test (CHO and V79 cells), REC-assay, UDS-test, chromosome aberration assay (CHO cells), mouse-micronucleus test (oral and i.p. application).
In a subchronic rat drinking water study no critical findings were obtained for NTN 33893-nitrosimine. The NOAEL was 100 ppm, equivalent to 13 mg/kg bw/day.

No synergistic or superadditive acute toxicity was seen in rats following simultaneous oral administration of imidacloprid with other insecticidal active ingredients such as cyfluthrin, methamidophos and flumethrin.

Human toxicological data

Based on data from periodical occupational examinations performed by the Bayer medical department, no adverse health effects have been reported for employees handling imidacloprid during the production of active ingredient and formulations. Mild cases of contact dermatitis in pet owners have been reported following use of veterinary formulations of imidacloprid [redacted] The effects are attributed to formulation-specific component(s) of this product not to imidacloprid itself.
From the experimental biological testing and from the field tests with imidacloprid formulations no negative effects on the health of the operators or workers were reported. No epidemiological studies on the general population are available. One case of accidental ingestion of approximately 10 mg imidacloprid per kg body weight by a four year old child did not result in signs of poisoning or adverse health effects.

Since there have been no documented cases of poisoning, information on symptoms or clinical signs in humans is not available. Based on agonistic effects of this class of compounds on the mammalian nicotinergic acetylcholine receptor, nicotine-like action may occur in severe intoxications. Complete recovery is expected within a few days. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning. A specific antidote is not known. In case of oral uptake, first aid measures should consist of removal of ingested compound by gastric lavage or induction of vomiting and symptomatic treatment. Contaminated skin should be washed immediately with plenty of water. Administration of activated charcoal was found to be effective under experimental conditions in laboratory rodents.

B.6.10.2 Calculation of the Acceptable Operator Exposure Level (AOEL)

For the calculation of the acceptable operator exposure level (AOEL) and a general risk assessment of imidacloprid for the operator, worker and bystander, the NOAELs of short-term toxicity studies as well as embryotoxicity studies will be taken into account. However, in case of accidental exposure, the acute toxicity values are of greatest importance. Imidacloprid has a moderate acute toxicity in rats after oral administration (oral LD_{50} ~500 mg/kg bw), did not induce toxicity after acute dermal or acute inhalatory exposure of an aerosol, is not irritant to eyes or skin and has no skin sensitising potential.

The short-term oral toxicity of imidacloprid is characterised by reduced body weight gains and sometimes an impairment of food conversion in rats, mice and dogs. Clinical chemistry and haematology data indicate effects on the liver, resulting in induction of hepatic microsomal enzymes and histological changes at lower doses and in functional impairments (reduction of cholesterol, triglycerides, total protein, albumin; increase of blood coagulation time, serum bilirubin) and cytotoxicity at higher doses. Liver weights were increased in rats and dogs.

In the embryotoxicity studies in rats and rabbits, decreased food consumption and body weight gains demonstrated maternal toxicity. Rabbits also showed mortality and complete litter losses from abortion or total litter resorption at the high dose level (72 mg/kg bw). In both species, effects on the foetuses (wavy ribs in rats; prenatal growth retardation and reduced ossification in rabbits) were only observed at dose levels which were toxic to the mothers.

Based on the findings described above, the NOAELs relevant for the derivation of an AOEL are listed in Table B.6.10-5.

Table B.6.10-5: NOAELs and LOAELs from oral short term and reproductive toxicity studies

Species	Study type	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	90-day range finding study	15 (120 ppm)	57 (600 ppm)
Rat	90-day study	14 (150 ppm)	61 (600 ppm)
Rat	90-day neurotoxicity study	10 (140 ppm)	63 (963 ppm)
Mouse	90-day study	400 (600 ppm)	2400 (1000 ppm)
Dog	28-day study	7.3 (200 ppm)	31 (1000 ppm)
Dog	90-day study	23.5 (200 ppm)	≤ 80 (1800/1200 ppm)
Dog	12-month study	15 (500 ppm)	70 (2500 ppm)
Rat	Embryotoxicity study	10	30
Rabbit	Embryotoxicity study	8	24

The lowest relevant NOAEL (15 mg/kg bw) in rats and dogs is based on effects on body weight gain, food consumption, liver weight and liver enzyme induction. A safety factor of 100 is considered appropriate. Taking into account a bioavailability of 100%, the acceptable operator exposure level is calculated to be:

AOEL = 15 mg/kg bw/day / 100 = 0.15 mg/kg bw/day

B.6.10.3 Calculation of the Acute Reference Dose (ARfD)

For the determination of the acute reference dose acute and short-term toxicity studies are considered to be important either because of a short overall treatment duration or because

adverse effects were observed within a few days from the start of the study or shortly after an acute high intake.

The oral LD_{50} of imidacloprid in rats was 500 mg/kg bw; signs of intoxication (apathy, respiratory problems) were observed at 100 mg/kg bw with gait impairment, tremors, spasms and convulsions occurring at higher doses. In the acute neurotoxicity study, a NOAEL for general systemic toxic effects after a single oral (gavage) administration to rats was achieved at a dose of 42 mg/kg bw. Effects observed at the LOAEL of 151 mg/kg bw included tremor, facial staining, reduced serum triglycerides and reduced motor/locomotor activity.

Trembling and severe tremor were also noted in the 28-day oral toxicity study in dogs on days after high substance intake (≥ 80 mg/kg bw) had occurred and in the 90-day oral toxicity study in dogs during the first week of treatment until week 5. The NOAEL for this endpoint from all available dog studies is considered to be 40 mg/kg bw.
Based on the above described findings, the relevant NOAELs are listed in Table B.6.10-6.

Table B.6.10-6: NOAELs and LOAELs from oral short term and reproductive toxicity studies

Species	Study type	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	Acute neurotoxicity study	42	151
Dog	28-day, 90-day and 1-year study	40*	70-80

* = overall NOAEL for tremors

The lowest relevant NOAEL is approximately 40 mg/kg bw/d in the rat and dog. The standard safety factor of 100 is considered appropriate for the calculation of the ARfD.
The ARfD is calculated to be:

ARfD = 40 mg/kg bw/day / 100 = 0.4 mg/kg bw/day.

B.6.10.4 Calculation of the Acceptable Daily Intake (ADI)

For the determination of the acceptable daily intake long-term toxicity studies and reproduction multigeneration toxicity studies are considered to be the most appropriate basis.

Long-term feeding studies in rats revealed effects on the thyroid (increased incidence of follicular colloid mineralisation) as the most sensitive parameter, indicating a treatment-related premature ageing of the gland without a concomittant effect on functional parameters. Males were more sensitive than females. In mice, an increased incidence of mineralisation in the thalamus was noted in both sexes at the highest dose level. No evidence of an oncogenic potential of imidacloprid was found in either the rat or the mouse long-term feeding studies.
The main finding in the two-generation study in rats consisted of reduced body weight gains in parent animals during the pre-mating treatment period and in offspring during the pre-weaning period.

The relevant NOAELs are shown in the table below:

Table B.6.10-7: Long-term NOAELs and LOAELs in mg/kg bw

Species	Study type	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)
Rat	24 month study	6 (100 ppm)	17 (300 ppm)
Mouse	24 month study	66 (330 ppm)	208 (1000 ppm)
Rat	Two-generation study	20 (250 ppm)	50 (700 ppm)

The NOAEL derived from the chronic toxicity and carcinogenicity study in rats is lower than any of the other studies. Therefore, this study with a NOAEL of 6 mg/kg bw will be used for the calculation of the acceptable daily intake (ADI). Using a conventional assessment factor of 100 the proposed ADI is

ADI = 6 mg/kg bw / 100 = 0.06 mg/kg bw.

B.6.11 Acute toxicity including irritancy and skin sensitisation of preparations (Annex IIIA 7.1)

The data for two preparations were submitted.

Confidor SL 200 (NTN 33893 200 SL) is an insecticide used in agriculture in numerous crops. It is formulated as a soluble concentrate (SL) containing nominal 200 g/L imidacloprid. During the past years the composition of Confidor SL 200 has been modified for product improvement purposes. An overview on the composition of the formulations used for toxicological testing and the formulation currently produced was given by the notifier. Formulation no. 03833/0047 has been tested for acute oral, dermal and inhalation toxicity and for skin and eye irritation and formulation no. 03833/0081 was used in the test for skin sensitisation. [redacted]

Gaucho FS 600 uncoloured (NTN 33893 600 FS) is a seed dressing liquid. It is formulated as a flowable concentrate (FS) containing nominal 600 g/L imidacloprid. With exception of testing for skin sensitisation, the formulation toxicity data of Gaucho FS 600 uncoloured are adopted from a nearly identical, but coloured formulation. The toxicity data established for the coloured product are valid also for the uncoloured formulation.

The results of the acute toxicity studies including irritancy and skin sensitisation are summarised in Table B.6.11-1 and Table B.6.11-2, respectively.

Table B.6.11-1: Acute toxicity of Confidor SL 200

Study	Species	Results	Reference
Acute oral*	Rat	LD_{50} (m): 2236 mg/kg bw LD_{50} (f): 2242 mg/kg bw	Bomann, W. (1990) TOX2003-2081
Acute dermal*	Rat	LD_{50} (m+f): > 5000 mg/kg bw	Bomann, W. (1990) TOX2003-2082
Acute inhalation* (head/nose only)	Rat	LC_{50} (m+f): > 6.312 mg/L air (4 h)	Maertins, T. (1990) TOX2003-2083
Skin irritation*	Rabbit	Not irritating	Maertins, T. (1990) TOX2003-2084
Eye irritation*	Rabbit	Not irritating	Maertins, T. (1990) TOX2003-2084
Skin sensitisation** (Buehler test; 3 ind.)	Guinea pig	Not sensitising	Diesing, L., Dreist, M. (1991) TOX2003-2085 (supplementary)

* Test substance: Formulation no. 03833/0047;
** Test substance: Formulation no. 03833/0081

Table B.6.11-2: Acute toxicity of Gaucho FS 600 uncoloured

Study	Species	Results	Reference
Acute oral*	Rat	LD_{50} (m+f): > 200 mg/kg bw < 2000 mg/kg bw	Bomann, W. (1995) TOX96-50611
Acute dermal*	Rat	LD_{50} (m+f): > 4000 mg/kg bw	Bomann, W. (1995) TOX96-50612
Acute inhalation* (nose only)	Rat	LC_{50} (m+f): > 1.862 mg/L air (4 h)	Pauluhn, J. (1996) TOX96-50613
Skin irritation*	Rabbit	Not irritating	Kroetlinger, F. (1994) TOX96-50614
Eye irritation*	Rabbit	Not irritating	Kroetlinger, F. (1994) TOX96-50614
Skin sensitisation (Maximisation Test)	Guinea pig	Sensitising	Vohr, H.-W. (2001) TOX2003-2097

* Test substance: Gaucho FS 600 Rot

Confidor SL 200 has a low acute oral, dermal and inhalation toxicity. The formulation is not irritating to skin and eyes. In a Buehler test with only 3 inductions and therefore considered only supplementary no skin sensitising properties in the guinea pig were seen. However, basing on the respective assessment of the active ingredient (M & K: not sensitising) and the co-formulants (no references to sensitising properties) the preparation is considered not to be sensitising.

Gaucho FS 600 uncoloured has a moderate acute oral toxicity with a LD_{50} > 200 mg/kg bw and < 2000 mg/kg bw and a low dermal and inhalation toxicity (at the highest tested aerosol concentration of nominal 5.0 mg/L, i.e. analytically 1.862 mg/L air, no mortality was observed). The formulation is not irritating to skin and eyes. In a Maximisation Test (M & K) skin sensitising properties in the guinea pig were seen.

In accordance with Directive 1999/45/EC on the basis of the results from acute toxicity testing, the following classification/labelling requirements are derived:

Confidor SL 200

Classification / Labelling:

No classification/labelling is needed.

Gaucho FS 600 uncoloured

Classification / Labelling:

Xn, Harmful; R 22 - Harmful if swallowed

R 43 - May cause sensitisation by skin contact

B.6.11.1 Acute toxicity of Confidor SL 200

B.6.11.1.1 Oral

Report: Bomann, W. (1990); TOX2003-2081:
NTN 33893 200 SL 03833/0047 imidacloprid (proposed) - Study for acute oral toxicity in rats. Bayer AG, unpublished report no.: 19499, date: 1990-09-10 (Date of work: February – April 1990).

GLP: Yes (certified laboratory)

Guideline: OECD 401; FIFRA § 81-1; EEC B 1

Deviations: None

Acceptability: The study is considered to be acceptable

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 074 B based on 03833/0047, content as: 207 g/L)
Test animals: 5 male and 5 female fasted Wistar rats per dose (Bor:WISW; SPF-Cpb)
Single administration of the test substance preparation (formulated in demineralised water) by gavage to 5 male and 5 female fasted Wistar rats at dose levels of 200 (only f), 250 (only m) 1600, 2000 and 2500 mg/kg bw, using an application volume of 10 mL/kg bw. The observation period lasted 14 days.

Findings:
Mortality was as follows: One females (10 %) at 1600 mg/kg bw; one male and two females (30 %) at 2000 mg/kg bw; four males and three females (70 %) at 2500 mg/kg bw (Table B.6.11-3).
The main clinical signs were apathy, reduced motility, labored breathing, in some cases spasms, periodic tremors and isolated cases of increased salivation and spastic gait.
Body weight loss occured in some animals in the lethal dose range which was compensated after the first week of the observation period.

Table B.6.11-3: Confidor SL 200 - acute oral toxicity

Dose (mg/kg bw)	Toxicological result*	Duration of signs	Time of death	Mortality (%)
Males				
250	0/0/5	--	--	0
1600	0/5/5	10 min – 3 d	--	0
2000	1/5/5	5 min – 3 d	3 h	20
2500	4/5/5	5 min – 5 d	2 h – 5 h	80
Females				
200	0/0/5	--	--	0
1600	1/5/5	5 min – 2 d	2 h	20
2000	2/5/5	10 min – 2 d	3 h – 1 d	40
2500	3/5/5	5 min – 5 d	2 h – 5 h	60

* Number of dead animals / number of animals with toxic signs / number of animal used

At the gross necropsy the following findings were observed in the animals which had died during the post-treatment observation period: lung: distended, patchy to dark; liver dark; bladder: full of clear urine; ulcerous foci and reddened mucous membrane in the glandular stomach; kidneys: pale. In the animals sacrified at the end of the observation period in one femal rat (dose: 200 mg/kg bw) a pale liver with clear lobulation was determined.

Conclusion:
The oral LD_{50} of Confidor SL 200 was found to be 2236 mg/kg bw for male and 2242 mg/kg bw for female rats. Therefore according to Directive 1999/45/EC no classification/labelling is needed.

B.6.11.1.2 Percutaneous

Report: Bomann, W. (1990); TOX2003-2082:
NTN 33893 200 SL 03833/0047 [c. n.: Imidacloprid (proposed)] - Study for acute dermal toxicity in rats. Bayer AG, unpublished report no.: 19532, date: 1990-09-20 (Date of work: February – May 1990).

GLP: Yes (certified laboratory)

Guideline: OECD 402, FIFRA § 81-2, EEC B 3

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 074 B based on 03833/0047, content as: 207 g/L)

Test animals: 5 male and 5 female fasted Wistar rats (Bor:WISW; SPF-Cpb)
The test material was applied to the intact dorsal skin of the animals for 24 hours. Each dose of the test article was weighed onto an aluminium foil (used to cover the skin site), separately for each animal. The test article was then mixed to a paste with cellulose powder (300 mg cellulose added to 1000 mg of the test article). The foil was fixed in place on the skin with an occlusive dressing. The animals were observed for 14 days.

Findings:
No mortality occurred (Table B.6.11-4). Apathy and periodic tremor were observed in one female at 5000 mg/kg bw. The male animals in this dose group exhibited a reddish coloration of the bedding at the urine sites.

Table B.6.11-4: Confidor SL 200 - acute dermal toxicity

Dose (mg/kg bw)	Toxicological result*	Duration of signs	Time of death	Mortality (%)
Males				
5000	0/0/5	--	--	0
Females				
2000	0/0/5	--	--	0
5000	0/1/5	2 d – 3 d	--	0

* Number of dead animals / number of animals with toxic signs / number of animal used;
Skin changes are not accounted for in this table.

Skin changes such as reddening and scabbing only occured in the female rats in the 5000 mg/kg bw dose group.
On day 4 of the test most animals showed losses in body weight which were generally compensated by the end of the post-treatment observation period.
At the gross necropsy at the end of the observation period no treatment-related findings were made.

Conclusion:
The dermal LD_{50} of Confidor SL 200 was found to be > 5000 mg/kg bw for male and female rats. Therefore no classification/labelling is needed.

B.6.11.1.3 Inhalation

Report: Maertins, T. (1990); TOX2003-2083:
NTN 33893 200 SL 03833/0047 – c. n.: Imidacloprid (proposed) - Acute inhalation toxicity in rats. Bayer AG, unpublished report no.: 19598, date: 1990-10-08 (Date of work: April 1990).

GLP: Yes (certified laboratory)

Guideline: OECD 403, FIFRA § 81-3, EEC B 2

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 074 B based on 03833/0047, content as: 207 g/L)
Test animals: 5 male and 5 female fasted Wistar rats per dose (Bor:WISW; SPF-Cpb)
Five male and five female Wistar rats per dose level were exposed (head/nose only) to the aerosolised test sample. The observation time was 14 days.

Findings:
The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of 1.24 µm. circa 91 % on a relative mass basis were ≤ 3 µm, which is within the respirable range.
There were no mortalities in the study (Table B.6.11-5).

Table B.6.11-5: Confidor SL 200 - acute inhalation toxicity

Group	Analytical concentration (mg/L air)	Toxicological result*	Duration of signs	Time of death
Males				
1	0	0/0/5	--	--
2	3.149	0/0/5	--	--
3	6.312	0/5/5	4 h – 1 d	--
Females				
1	0	0/0/5	--	--
2	3.149	0/0/5	--	--
3	6.312	0/5/5	4 h – 1 d	--

* Number of dead animals / number of animals with toxic signs / number of animal used

All animals of group 3 displayed transient nonspecific signs as ungroomed fur, piloerection, reduced motility and reduction in body weight.

Conclusion:
The inhalation LC_{50} (4 h) of Confidor SL 200 was found to be > 6.312 mg/L air for male and female rats.
Therefore, no classification/labelling is needed.

B.6.11.1.4 Skin irritation

Report: Maertins, T. (1990); TOX2003-2084:
NTN 33893 200 SL 03833/0047 (c. n.: Imidacloprid, proposed) – Study for skin and eye irritation/corrosion in rabbits. Bayer AG, unpublished report no.: 19035, date: 1990-05-04 (Date of work: February - March 1990).

GLP: Yes (certified laboratory)

Guideline: OECD 404, EEC B 4

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 074 B based on 03833/0047, content as: 207 g/L)
Test animals: Three female New Zealand White rabbits (HC:NZW)
The undiluted liquid test substance mixture (0.5 mL) was applied dermally to the intact skin of three New Zealand White rabbits for 4 hours under a semiocclusive dressing. After the patches were removed the treated area was rinsed with water. The animals were observed for skin irritation at 1 h, 24 h, 48 h and 72 h and on day 7 after removal of the patch.
Different sites on the same animals were used as controls.

Findings:
Skin findings are summarised in Table B.6.11-6.

Table B.6.11-6: Skin irritation scores (erythema/oedema)

Animal number	DRAIZE grade after patch removal					Mean scores
	1 h	24 h	48 h	72 h	7 d	24-48-72 h
1	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0.0 / 0.0
2	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0.0 / 0.0
3	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0.0 / 0.0

The test substance did not induce any skin reactions.

Conclusion:
Confidor SL 200 is non-irritating to skin. Therefore, no classification/labelling is needed.

B.6.11.1.5 Eye irritation

Report: Maertins, T. (1990); TOX2003-2084:
NTN 33893 200 SL 03833/0047 (c. n.: Imidacloprid, proposed) – Study for skin and eye irritation/corrosion in rabbits. Bayer AG, unpublished report no.: 19035, date: 1990-05-04 (Date of work: February - March 1990).

GLP: Yes (certified laboratory)

Guideline: OECD 405, EEC 92/69 B 5

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 074 B based on 03833/0047, content as: 207 g/L)
Test animals: Six female New Zealand White rabbits (HC:NZW)
The unchanged test substance was applied in a single dose of 0.1 mL to the right conjunctival sac (the left eye served as a control) of six New Zealand White rabbits. Readings were carried out at 1, 24, 48 and 72 hours and on day 7 after application of the test substance.

Findings:
The eye irritation scores are given in Table B.6.11-7.

Table B.6.11-7: Eye irritation: readings averaged over 24, 48 and 72 hrs

Time after administration	Cornea (opacity)	Iris (iritis)	Conjunctivae	
			(redness)	(chemosis)
Animal no. 1/2/3/4/5/6				
1 h	0/1/0/0/0/1	0/0/0/0/0/0	1/1/1/1/1/1	1/1/3/1/0/1
24 h	0/0/0/0/0/0	0/0/0/0/0/1	2/2/2/1/1/2	0/1/1/1/0/2
48 h	0/0/0/0/0/0	0/0/0/0/0/0	2/2/2/1/1/2	0/1/1/0/0/0
72 h	0/0/0/0/0/0	0/0/0/0/0/0	0/1/0/1/0/0	0/0/0/0/0/0
7 d	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0	0/0/0/0/0/0

Exposure of the test substance to the eye caused slight reactions of the mucous membranes and the cornea which proved to be fully reversible within 7 days.

Conclusion:
Confidor SL 200 is slightly irritating to the eye. However, the criteria according to Directive 1999/45/EC were not fulfilled and no classification/labelling is needed.

B.6.11.1.6 Skin sensitisation

Report: Diesing, L. and Dreist, M. (1991); TOX2003-2085: NTN 33893 200 SL 03833/0081 [c. n.: Imidacloprid, (proposed)] – Studies on skin sensitising effects in guinea pigs (Buehler test). Bayer AG, unpublished report no.: 20456, date: 1991-07-16 (Date of work: March – April 1991).

GLP: Yes (certified laboratory)

Guideline: OECD 406, FIFRA § 81-6, EEC B 6

Deviations: None.
At an ECCO-meeting in 1998 it was stated that the Buehler-test with 3 applications/inductions is not a valid test to assess skin sensitisation. Instead of this, the Magnusson & Kligman-test or the Buehler test with 9 applications were considered appropriate.

Acceptability: The study is considered to be supplementary.

Material and Methods:
Test material: NTN 33893 200 SL (formulation no. 099 E based on 03833/0081, content as: 210.6 g/L)
Test animals: 41 male guinea pigs (Bor:DHPW; SPF), including range finding study
In the preliminary range-finding study a 50 % test article formulation (diluted with sterile physiological saline solution) was determined as the minimal irritant concentration. Accordingly, this concentration was used for the 1st to 3rd induction exposure. The maximum non-irritant concentration for the challenge was determined to be a 25 % test article formulation. However, after induction with a 50 % test article formulation only one animal in the test article group showed slight skin reddening in places following the second induction. No skin reactions occured in the control groups. The 50 % test article formulation must also have been a threshold concentration as far as skin irritant effect was concerned. For this reason a 25 % and 50 % test article formulation was applied during the challange.

Findings:
Following a challange with the 50 % test article formulation only one animal (8.3 %) of the test article group exhibited slight skin redness in places. This only occured after 48 h and not after 24 h or 72 h. Accordingly, this was regarded as a coincidental finding which has no connection with a sensitising effect on the study animal. No skin reactions occured in either the control or test article group following application of the 25 % test article formulation.

Conclusion:
Confidor SL 200 has not shown skin-sensitisation potential under the conditions of the Buehler test with only 3 inductions. However, this test is not considered a valid test to assess skin sensitisation (see deviations).

B.6.11.2 Acute toxicity of Gaucho FS 600 uncoloured

With exception of testing for skin sensitisation, the formulation toxicity data of Gaucho FS 600 uncoloured are adopted from a nearly identical, but coloured formulation, "Gaucho FS 600 Rot". The toxicity data established for the coloured product are valid also for the uncoloured formulation.

B.6.11.2.1 Oral

Report:	Bomann, W. (1995); TOX96-50611: NTN 33893 600 FS 03905/0711 [c. n.: Imidacloprid (proposed)] - Study for acute oral toxicity in rats. Bayer AG, unpublished report no.: 24128, date: 1995-07-04 (Date of work: September – November 1994).
GLP:	Yes (certified laboratory)
Guideline:	OECD 401; FIFRA § 81-1; EEC B 1
Deviations:	None
Acceptability:	The study is considered to be acceptable

Material and Methods:
Test material: GAUCHO FS 600 ROT = NTN 33893 600 FS (formulation no. 0731 based on 03905/0711, content as: 601.4 g/L)
Test animals: 5 male and 5 female fasted Wistar rats per dose (Hsd/Win:WU; SPF)
Single administration of the test substance preparation (formulated in demineralised water) by gavage to 5 male and 5 female fasted Wistar rats at dose levels of 200 and 2000 mg/kg bw, using an application volume of 10 mL/kg bw. The observation period lasted 15 days.

Findings:
100 % mortality was observed at 2000 mg/kg bw (Table B.6.11-8). At doses of 200 mg/kg bw and above clinical signs were observed. They were as follows: decreased motility and reactivity, labored breathing, narrowed palpebral fissure, transient palmospasm and red colored feces.
No treatment related effects in body weight and body weight gain were seen.
The gross pathology investigations performed on animals which died intercurrently afforded the following effects: residues of test substance in duodenum, jejunum and stomach; liver and lung dark red discolorations; spleen light pale discoloration. No pathological changes were observed in animals sacrified at the end of the observation period.

Table B.6.11-8: Gaucho FS 600 Rot - acute oral toxicity

Dose (mg/kg bw)	Toxicological result*	Duration of signs	Time of death	Mortality (%)
Males				
200	0/3/5	2 d – 3 d	--	0
2000	5/5/5	20 min – 2 h	50 min – 2 h	100
Females				
200	0/3/5	2 d – 3 d	--	0
2000	5/5/5	30 min – 2 h	1 h – 2 h	100

* Number of dead animals / number of animals with toxic signs / number of animal used

Conclusion:
The oral LD_{50} of Gaucho FS 600 Rot was found to be > 200 mg/kg bw and < 2000 mg/kg bw for male and female rats. The respective classification/labelling (R 22) is needed for Gaucho FS 600 uncoloured, too.

B.6.11.2.2 Percutaneous

Report: Bomann, W. (1995) TOX96-50612:
NTN 33893 600 FS 03905/0711 [c. n.: Imidacloprid (proposed)] - Study for acute dermal toxicity in rats. Bayer AG, unpublished report no.: 23946, date: 1995-04-24 (Date of work: October – November 1994).

GLP: Yes (certified laboratory)

Guideline: OECD 402, FIFRA § 81-2, EEC B 3

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: GAUCHO FS 600 ROT = NTN 33893 600 FS (formulation no. 0731 based on 03905/0711, content as: 601.4 g/L)
Test animals: 5 male and 5 female fasted Wistar rats per dose (Hsd/Win:WU; SPF)
The test material was applied to the intact dorsal skin of the animals for 24 hours. Each dose of the test article was weighed onto an aluminium foil (used to cover the skin site), separately for each animal. The test article was then mixed to a paste with cellulose powder (150 mg cellulose added to 1000 mg of the test article). The foil was fixed in place on the skin with an occlusive dressing. The animals were observed for 14 days.

Findings:
No mortality occurred and no clinical signs were observed (Table B.6.11-9). There were no local findings at the treatment site.

Table B.6.11-9: Gaucho FS 600 Rot - acute dermal toxicity

Dose (mg/kg bw)	Toxicological result*	Duration of signs	Time of death	Mortality (%)
Males				
2000	0/0/5	--	--	0
4000	0/0/5	--	--	0
Females				
4000	0/0/5	--	--	0

* Number of dead animals / number of animals with toxic signs / number of animal used;

At the gross necropsy at the end of the observation period no treatment-related findings were made.

Conclusion:
The dermal LD_{50} of Gaucho FS 600 Rot was found to be > 4000 mg/kg bw for male and female rats. Therefore no classification/labelling is needed for Gaucho FS 600 uncoloured, too.

B.6.11.2.3 Inhalation

Report: Pauluhn, J. (1996); TOX96-50613:
NTN 33893 600 FS 03905/0711 (c. n.: Imidacloprid, proposed) - Study on acute inhalation toxicity in rats according to OECD No. 403. Bayer AG, unpublished report no.: 24578, date: 1996-01-02 (Date of work: September 1994).

GLP: Yes (certified laboratory)

Guideline: OECD 403, FIFRA § 81-3, EEC B 2

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: GAUCHO FS 600 ROT = NTN 33893 600 FS (formulation no. 0731 based on 03905/0711, content as: 601.4 g/L)
Test animals: 5 male and 5 female fasted Wistar rats per dose (Hsd/Win:WU; SPF)
The test article was administered as an aerosol under nose only exposure conditions. The animals were exposed for four hours to concentrations of 0.274 and 1.862 mg/L air. The observation time was 14 days.

Findings:
The particle size distribution revealed a mass median aerodynamic diameter (MMAD) of ca. 2.5 µm. Ca 50 % on a relative mass basis were ≤ 3 µm, which is within the respirable range.
There were no mortalities in the study (Table B.6.11-10).

Table B.6.11-10: Gaucho FS 600 uncoloured - acute inhalation toxicity

Group	Analytical concentration (mg/L air)	Toxicological result*	Duration of signs	Time of death
Males				
1	0	0/0/5	--	--
2	0.274	0/0/5	--	--
3	1.862	0/5/5	2 d	--
Females				
1	0	0/0/5	--	--
2	0.274	0/0/5	--	--
3	1.862	0/5/5	1 d	--

* Number of dead animals / number of animals with toxic signs / number of animal used

Exposure to 1.862 mg/L was accompanied with transiently occuring and mild to moderate signs such as laboured breathing pattern, decreased motility and muscle tone, ungroomed hair coat and piloerection. No treatment-releated gross pathological changes were observed in either sex. As recorded by the notifier the concentration of 1.862 mg/L air was achieved by the nebulisation of a 50 % (w/v) aqueous solution and meets the limit concentration of 2mg/L (SOT, 1992).

Conclusion:
The inhalation LC_{50} (4 h) of Gaucho FS 600 Rot was found to be > 1.862 mg/L air (nominal: > 5.0 mg/L air) for male and female rats. Therefore no classification/labelling is needed for Gaucho FS 600 uncoloured, too.

B.6.11.2.4 Skin irritation

Report: Kroetlinger, F. (1994); TOX96-50614: NTN 33893 600 FS 03905/0711 [c. n.: Imidacloprid (proposed)] – Study for skin and eye irritation/corrosion in rabbits. Bayer AG, unpublished report no.: 23289, date: 1994-08-29 (Date of work: July - August 1994).

GLP: Yes (certified laboratory)

Guideline: OECD 404, EEC B 4

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: GAUCHO FS 600 ROT = NTN 33893 600 FS (formulation no. 0731 based on 03905/0711, content as: 601.4 g/L)
Test animals: Three male New Zealand White rabbits (HC:NZW)
The undiluted liquid test substance mixture (0.5 mL) was applied dermally to the intact skin of three New Zealand White rabbits for 4 hours under a semiocclusive dressing. After the patches were removed the treated area was rinsed with water. Skin irritation was scored and recorded at 1, 24, 48 and 72 hours after removal of the patch. The observation time was 7 days. Different sites on the same animals were used as controls.

Findings:
Skin findings are summarised in Table B.6.11-11. In all three rabbits the evaluation of skin erythema was not possible due to an intense colouration of the treatment sites induced by the test substance. Nevertheless, no other inflammatory signs (eschar and oedema formation) became apparent within the observation period of 7 days. This evidence indicates no hazard potential to the skin and the test substance may therefore be regarded as not irritating to the skin.

Table B.6.11-11: Skin irritation scores (erythema/oedema)

Animal number	Toxicological results: DRAIZE grade after							
	1 h		24 h		48 h		72 h	
	E	O	E	O	E	O	E	O
1	#	0	#	0	#	0	#	0
2	#	0	#	0	#	0	0	0
3	#	0	#	0	#	0	#	0

E = Erythema and eschar formation, O = Oedema formation; 0 = no pathological findings,
# = exposed skin areas stained in colour of the test substance, evaluation of erythema not possible

Conclusion:
Gaucho FS 600 Rot is not irritating to skin. Therefore no classification/labelling is needed for Gaucho FS 600 uncoloured, too.

B.6.11.2.5 Eye irritation

Report: Kroetlinger, F. (1994); TOX96-50614: NTN 33893 600 FS 03905/0711 [c. n.: Imidacloprid (proposed)] – Study for skin and eye irritation/corrosion in rabbits. Bayer AG, unpublished report no.: 23289, date: 1994-08-29 (Date of work: July - August 1994).

GLP: Yes (certified laboratory)

Guideline: OECD 405, EEC B 5

Deviations: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: GAUCHO FS 600 ROT = NTN 33893 600 FS (formulation no. 0731 based on 03905/0711, content as: 601.4 g/L)
Test animals: Three male New Zealand White rabbits (HC:NZW)
The unchanged test substance was applied in a single dose of 0.1 mL to the conjunctival sac of one eye of each of three New Zealand White rabbits. The other eye remained untreated and served as control. 24 hours after instillation of the test substance the treated eye was rinsed with normal saline. Readings were carried out at 1, 24, 48 and 72 hours after application of the test substance.

Findings:
The eye irritation scores (DRAIZE grades) are given in Table B.6.11-12.

Table B.6.11-12: Eye irritation: DRAIZE grades

Time after administration	Cornea (opacity)	Iris (iritis)	Conjunctivae	
			(redness)	(chemosis)
Animal no. 1/2/3				
1 h	0/0/0	0/0/0	0/0/0	0/0/0
24 h	0/0/0	0/0/0	0/0/0	0/0/0
48 h	0/0/0	0/0/0	0/0/0	0/0/0
72 h	0/0/0	0/0/0	0/0/0	0/0/0

The test substance did not induce any reactions of the eyes.

Conclusion:
Gaucho FS 600 Rot is not irritating to eyes. Therefore no classification/labelling is needed for Gaucho FS 600 uncoloured, too.

B.6.11.2.6 Skin sensitisation

Report: Vohr, H.-W.(2001); TOX2003-2097:
NTN 33893 600 FS Study for the skin sensitisation effect in guinea pigs (Guinea pig Maximisation Test according to Magnusson and Kligman), Bayer AG, unpublished report no.: 30820, date: 2001-03-15 (Date of work: October - November 2000).

GLP: Yes (certified laboratory)

Test Method: OECD 406, OPPTS 870.2600, EEC B 6

Deviatons: None

Acceptability: The study is considered to be acceptable.

Material and Methods:
Test material: GAUCHO FS 600 UNCOLOURED = NTN 33893 600 FS (Batch no. 233926415), purity: 60.7 %)
Test animals: 37 female guinea pigs (Hsd/Poc:DH); SPF – including range-finding tests
Gaucho FS 600 uncoloured was tested for its sensitising effect on the skin of the guinea pig in the Guinea Pig Maximisation Test. The study was performed in 20 guinea pigs in the test group and 10 animals in the control group.
On the basis of a pretest, intradermal induction was performed using a 5 % solution of the test article in sterile physiological saline solution to yield an emulsion. The epicutaneous induction and the challenge were performed using 100 % test article.

Findings:
After the intradermal induction the animals in the control group and in the test group showed strong effects up to encrustation at the injection sites. After removal of the patch of the second induction, the treatment area showed no skin reactions.
The challenge with the 100 % test concentration led to skin effects (grade 1) in 11 of 20 animals (55 %) in the test group and no skin effects were seen in the control group animals (Table B.6.11-13).

Table B.6.11-13: Number of animals exhibiting skin effects

	Test group (20 animals)				Control group (10 animals)			
	Test item patch		Control patch		Test item patch		Control patch	
Hours	48	72	48	72	48	72	48	72
Challenge 100 %	11	0	0	0	0	0	0	0

Conclusions:
Gaucho FS 600 uncoloured has shown skin-sensitisation potential under the conditions of the Maximisation test according to Magnusson and Kligman and has to be classified accordingly (R 43).

B.6.12 Dermal absorption (Annex IIIA 7.3)

No experimental dermal absorption data are available for imidacloprid. Therefore, the assessment is based on physico-chemical endpoints. The values for molecular mass, MW = 255.7, and $\log P_{ow} = 0.57$ justify the assumption of 100 % dermal absorption for imidacloprid. The actual dermal absorption is likely to be lower as acute and subacute dermal toxicity studies in rats and rabbits, respectively, did not produce any signs of intoxication at doses which would have resulted in severe toxicity after oral administration.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

Besides its active ingredient imidacloprid, the preparations Confidor SL 200 (NTN 33893 200 SL) and Gaucho FS 600 uncoloured (NTN 33893 600 FS) contains different co-formulants. The respective data are given in Safety Data Sheets. The possible acute toxic properties of all co-formulants are covered by the studies with the preparations.

B.6.14 Exposure data (Annex IIIA 7.2)

B.6.14.1 Confidor SL 200

Confidor SL 200 is an insecticide containing the active substance imidacloprid (200 g/L). It is applied to high crops in orchards (apples) with tractor mounted air blast sprayers and to field crops (tomatoes) using either tractor mounted sprayers with hydraulic boom or hand held sprayers. It is also used with hand held spray equipment to treat tomatoes in greenhouses.

B.6.14.1.1 Operator exposure

B.6.14.1.1.1 Estimation of operator exposure; risk assessment

The operator exposure estimates are calculated using both the German model and the UK-POEM: For greenhouse application operator exposure was estimated with a GLP study performed for the generic assessment of products used in greenhouses.

- Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protections); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirschaft, Berlin-Dahlem, n° 277, 1992;

- Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators (UK MAFF) 1986 and the Predictive Operator Exposure Model (POEM) (UK MAFF) 1992.

- Mich, G. (1996): Operator Exposure in Greenhouses During Practical Use of Plant Protection Products; Project EF 94-02-03; june 6, 1996; ECON GmbH Ingelheim, conducted in Germany under the sponsorship of IVA (Industrieverband Agrar): “Greenhouse-study”

The assessed scenarios are summarised in Table B.6.14-1.

Table B.6.14-1: Scenarios/use conditions for the exposure calculation

Crop and Technique	Treated surface per working day	Max. use rate		max. in-use concentration (mg as/mL)	Models used
		kg as/ha	product: L/ha		
Apple Air blast sprayer	8 ha	0.105	(0.53)	–	German model
	15 ha	0.105	0.53	0.07	UK-POEM
Apple Hand held sprayer	1 ha	0.105	(0.53)	–	German model
Tomato Tractor boom sprayer	20 ha	0.1	(0.5)	–	German model
	30 ha	0.1	0.5	0.1	UK-POEM
Tomato* Hand held sprayer	1 ha	0.1	0.5	-	-
Tomato Hand held sprayer (greenhouse)	1	0.15	(0.75)	–	German model; "Greenhouse-study

* see calculation /assessment for apples: Hand held sprayer (only marginal differences in the use rate)

Estimates of operator exposure under field conditions

Where model data are available operator exposure estimates are calculated using both the German model and the UK-POEM. In cases of data gaps in a model, only the model containing data for the relevant scenario is taken.

Estimated operator exposure using the German model

The following assumptions are made for the estimation of operator exposure:

Formulation type: SL
Dermal absorption rate: 100 % (B.6.12)
Body weight of an operator: 70 kg

Using the input parameters and the scheme of the calculation model (Appendix 1-3), the estimated operator exposure can be calculated for mixing/loading (m/l) and application (appl.). The results for the estimated dermal and inhalation exposures are given in Table B.6.14-2, Table B.6.14-3 and Table B.6.14-4. The calculations were carried out for different conditions:

Scenario 1: No PPE, disregarding the recommendations on the label, no protective equipment used when handling the undiluted product and during application.

Scenario 2: PPE: gloves, standard protective garment and sturdy footwear used when handling the undiluted product (handling of product during mixing/loading).

Scenario 3: PPE: gloves, standard protective garment and sturdy footwear used when handling the undiluted and the diluted product (handling of product during mixing/loading and application).

Table B.6.14-2: Estimated operator exposure using the German model for apple, air blast sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/d)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl. garment: appl.)
Dermal exposure			
– Mixing/loading	2.016	0.020	0.020
– Application	9.660	9.660	1.417
– Total, dermal	11.676	9.680	1.437
Inhalation exposure			
– Mixing/loading	0.00050	0.00050	0.00050
– Application	0.01512	0.01512	0.01512
– Total, inhalation	0.01562	0.01562	0.01562
Total exposure: (dermal + inhalation)	**11.6916** (0.1670 mg/kg bw/d)	**9.6956** (0.1385 mg/kg bw/d)	**1.4526** (0.0208 mg/kg bw/d)

Table B.6.14-3: Estimated operator exposure using the German model for apple, hand held sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/d)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl. garment: appl.)
Dermal exposure			
– Mixing/loading	21.525	0.21525	0.21525
– Application	4.242	4.24200	0.64638
– Total, dermal	25.767	4.45725	0.86163
Inhalation exposure			
– Mixing/loading	0.00525	0.00525	0.00525
– Application	0.03150	0.03150	0.03150
– Total, inhalation	0.03675	0.03675	0.03675
Total exposure: (dermal + inhalation)	**25.8038** (0.3686 mg/kg bw/d)	**4.4940** (0.0642 mg/kg bw/d)	**0.8984** (0.0128 mg/kg bw/d)

Table B.6.14-4: Estimated operator exposure using the German model for tomatoes, tractor boom sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/d)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl. garment: appl.)
Dermal exposure			
– Mixing/loading	4.80	0.048	0.0480
– Application	4.08	4.080	0.2876
– Total, dermal	8.88	4.128	0.3356
Inhalation exposure			
– Mixing/loading	0.0012	0.0012	0.0012
– Application	0.0020	0.0020	0.0020
– Total, inhalation	0.0032	0.0032	0.0032
Total exposure: (dermal + inhalation)	**8.8832** (0.1269 mg/kg bw/d)	**4.1312** (0.0590 mg/kg bw/d)	**0.3388** (0.0048 mg/kg bw/d)

Using the German model without PPE, the estimated exposures are calculated to be 11.9616 mg/person/d for apples (air blast sprayer), 25.8038 mg/person/d for apples (hand held sprayer) and 8.8832 mg/person/d for tomatoes (tractor boom sprayer). PPE reduces these values respectively.

Estimated operator exposure using the UK model

The following assumptions are made for the estimation of operator exposure:

Formulation type:	SL
Packaging:	Calculation with 5 L (wide neck)
Dermal absorption rate:	100 % (B.6.12)
Body weight of an operator:	60 kg

Using the input parameters and the scheme of the calculation model, the estimated operator exposure can be calculated for mixing/loading and application (Appendix 4 – 9). The results for the estimated dermal and inhalation exposures are given in Table B.6.14-5 and Table B.6.14-6. The calculations were carried out for different conditions, as recommended by the notifier:

Scenario 1: No PPE, disregarding the recommendations on the label, no protective equipment used when handling the undiluted product and during application

Scenario 2: PPE: gloves only during mixing/loading (5 % penetration)

Scenario 3: PPE: gloves during mixing/loading (5 % penetration) and during spray application (10 % penetration)

Table B.6.14-5: Estimated operator exposure using the UK-POEM for apple, air blast sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/day)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.)
Dermal exposure			
– Mixing/loading	4.0000	0.2000	0.2000
– Application	8.4840	8.4840	5.9640
– Total, dermal	12.4840	8.6840	6.1640
Inhalation exposure			
– Mixing/loading	-	-	-
– Application	0.0210	0.0210	0.0210
– Total, inhalation	0.0210	0.0210	0.0210
Total exposure: (dermal + inhalation)	**12.5050** (0.2084 mg/kg bw/d)	**8.705** (0.1451 mg/kg bw/d)	**6.1850** (0.1031 mg/kg bw/d)

Table B.6.14-6: Estimated operator exposure using the UK-POEM for tomatoes, tractor boom sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/day)		
	Scenario 1 (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.)
Dermal exposure			
– Mixing/loading	6.0000	0.3000	0.3000
– Application	4.1550	4.1550	0.6450
– Total, dermal	10.1550	4.4550	0.9450
Inhalation exposure			
– Mixing/loading	-	-	-
– Application	0.0060	0.0060	0.0060
– Total, inhalation	0.0060	0.0060	0.0060
Total exposure: (dermal + inhalation)	**10.1610** (0.1694 mg/kg bw/d)	**4.4610** (0.0744 mg/kg bw/d)	**0.9510** (0.0159 mg/kg bw/d)

Using the UK-POEM without PPE, the estimated exposures are calculated to be 12.5050 mg/person/d for apples (air blast sprayer) and 10.1610 mg/person/d for field tomatoes (tractor boom sprayer). PPE reduces these values respectively.

Estimates of operator exposure in greenhouse tomatoes

Neither the German model nor the UK-POEM provide relevant data to assess the exposure of operators for greenhouses applications. Operator exposure was therefore estimated with a GLP study performed for the generic assessment of products used in greenhouses:
A summary of the study design and the results is briefly presented below:
Several scenarios were investigated:

a. mixing/loading of a WP for knapsack-application
b. application in low crops on tables
c. application in high crops (roses, height up to 1.8 m)
d. airborne concentrations after application

The products - selected as typical for greenhouse applications - were: Euparen WP (Dichlofluanid), Rody (Fenpropathrin) and Saprol Neu (Triforine). Exposure measurements were performed using passive dosimetry techniques.

Dermal exposure of the body was determined via patches as well as by analysing whole body underwear. The results of the patches correspond to potential dermal exposure whereas the results of the underwear can be regarded as actual dermal exposure when wearing only one layer of clothing. Hand exposure was measured via glove rinsings (potential dermal exposure) and hand rinsings (actual dermal exposure). For the determination of head exposure a patch was fixed to a cap.

Inhalation exposure was measured via adsorbent tubes fixed to the garments at the height of the operator's mouth and connected to a personal battery powered pump.
Samples were extracted for analysis followed by gas chromatographic determination. The results of the measurement are reported as determined (i.e. µg as per sample) and as specific exposures, i.e. as mg of exposure per kg of as handled. The latter facilitates the use of the data for generic purposes.

The study provides data on mixing/loading of WPs only. Therefore, generic exposure estimates were taken from the EU wide accepted German model.

Mixing/loading in greenhouses can be performed in two ways:
- mixing and loading a knapsack sprayer for each application separately or
- mixing and loading a large tank to which the spray equipment is then connected by a hose. This scenario can be compared to mixing/loading of tractor-mounted tanks.
As long as only small plots are treated the “knapsack procedure” is likely to be performed; however, as soon as larger areas are going to be treated this procedure is much too time consuming and the use of a large tank will be the usual practice.

The results of scenario C of the generic study are used to assess the exposure to imidacloprid during application in greenhouses. The application to high crops can be regarded as a worst case compared to an application to low crops on tables.

Table B.6.14-7: Specific exposure during application of high crops in greenhouse, Scenario C, IVA-study

Route of exposure during application	Actual exposure (i.e. with PPE)	Potential exposure (i.e. without PPE)
	(mg as/kg as handled)	
Inhalation exposure		**0.108**
Dermal exposure: head		**1.56**
Dermal exposure: hands	**0.008**	**13.2**
Body = half of upper arms, forearms, thighs, lower legs	0.192	70.0
+ trunk = half of upper arms, chest, back	0.036	12.5
Dermal exposure: whole body	**0.228**	**82.5**

The following assumptions are made for the estimation of operator exposure:

Formulation type:	SL
Dermal absorption rate:	100 % (B.6.12)
Body weight of an operator:	60 kg
Application rate:	0.15 kg as/ha
Treated area:	1 ha/d

Using the data from the German model for mixing/loading and the generic data from the Greenhouse-study for application the estimated operator exposure can be calculated for knapsack sprayer and for spray equipment connected with a large tank. (Appendix 10 – 11). The results for the estimated dermal and inhalation exposures are given in Table B.6.14-8 and Table B.6.14-9. The calculations were carried out for two different conditions:

Scenario 1: No PPE (according to the conditions in the Greenhouse-study)

Scenario 2: With PPE: - during mixing/loading: gloves (1 % penetration)
- during application PPE according to the conditions in the Greenhouse-study

Table B.6.14-8: Estimated operator exposure in greenhouses: Knapsack sprayer

Exposure route and type of work	Estimated operator exposure (mg/person/d)	
	Scenario 1 (no PPE)	**Scenario 2** (with PPE)
Dermal exposure		
– Mixing/loading	30.75	0.3075
– Application	14.589	0.2694
– Total, dermal	45.339	0.5769
Inhalation exposure		
– Mixing/loading	0.0075	0.0075
– Application	0.0162	0.0162
– Total, inhalation	0.0237	0.0237
Total exposure: (dermal + inhalation)	**45.3627** (0.7560 mg/kg bw/d)	**0.6006** (0.0100 mg/kg bw/d)

Table B.6.14-9: Estimated operator exposure in greenhouses: Hand held spraying with spray lance connected to stationary large tank

Exposure route and type of work	Estimated operator exposure (mg/person/d)	
	Scenario 1 (no PPE)	**Scenario 2** (with PPE)
Dermal exposure		
– Mixing/loading	0.3600	0.0036
– Application	14.5890	0.2694
– Total, dermal	14.9490	0.237
Inhalation exposure		
– Mixing/loading	0.00009	0.00009
– Application	0.0162	0.0162
– Total, inhalation	0.0163	0.0163
Total exposure: (dermal + inhalation)	**14.9653** (0.2494 mg/kg bw/d)	**0.2893** (0.0048mg/kg bw/d)

The estimated operator exposure in greenhouses (without PPE) are calculated to be 45.3627 mg/person/d for knapsack sprayers and 14.9653 mg/person/d if stationary large tanks were used. PPE reduces these values respectively.

Comparison of estimated and tolerable exposures

Since the dermal absorption rate is assumed to be 100 % (B.6.12) the systemic exposure is identical with the calculated exposure as given in Table B.6.14-2 to Table B.6.14-6 and Table B.6.14-8 to Table B.6.14-9 as "total exposure", respectively.

The calculated systemic exposure is compared with the proposed systemic AOEL of 0.15 mg/kg bw/d (see B.6.10.2). The values are given in Table B.6.14-10.

Table B.6.14-10: Results of the model calculations and a comparison with the proposed systemic AOEL

		Systemic exposure* (mg/kg bw/d)	**% of AOEL syst.** (0.15 mg/kg bw/d)
Apple			
Tractor mounted air blast sprayer			
German model	no PPE	0.1670	111.3
	gloves: m/l	0.1385	92.3
	gloves: m/l and appl. garment: appl.	0.0208	13.8
UK-POEM	no PPE	0.2084	138.9
	gloves: m/l	0.1451	96.7
	gloves: m/l and appl.	0.1031	68.7
Hand held sprayer			
German model	no PPE	0.3686	245.8
	gloves: m/l	0.0642	42.8
	gloves: m/l and appl. garment: appl.	0.0128	8.62
Tomato (field)**			
Tractor mounted boom sprayer			
German model	no PPE	0.1269	84.6
	gloves: m/l	0.0590	39.3
	gloves: m/l and appl. garment: appl.	0.0048	3.22
UK-POEM	no PPE	0.1694	112.9
	gloves: m/l	0.0744	49.6
	gloves: m/l and appl.	0.0159	10.6
Tomato (greenhouse)			
Knapsack sprayer	no PPE	0.7560	504.0
	with PPE	0.0100	6.7
Spray lance with stationary tank	no PPE	0.2494	166.3
	with PPE	0.0048	3.2

* See B.6.14-2 to B.6.14-6 and B.6.14-8 to B.6.14-9 "total exposure".; in the calculations a body weight of 70 kg (German model) or 60 kg (UK-POEM and Greenhouse-study) and a dermal absorption rate of 100 % is used.

** For hand held sprayer see calculation /assessment for apples (only marginal differences in the use rate)

Conclusion:
Assuming a dermal absorption rate of 100 %, on the basis of the German model and the UK-POEM without PPE, the estimated systemic exposure for operators using Confidor SL 200 in apples and tomatoes accounts for up to 246 % of the proposed systemic AOEL. By wearing PPE (gloves during mixing/loading), the exposure can be reduced to values not exceeding the proposed AOEL. Using the data from the German model for mixing/loading and the generic data from the Greenhouse-study for application the estimated operator exposure for knapsack sprayers and for using an spray equipment connected with a large tank was calculated to be 7 % and 3 % of the systemic AOEL, respectively, if wearing of PPE was considered into account.

B.6.14.1.1.2 Measurement of operator exposure

Since the risk assessment carried out indicates that the health-based limit value (systemic AOEL) will not be exceeded under practical conditions of use (with PPE), a study to provide a measurement of operator exposure to imidacloprid, the active ingredient of Confidor SL 200, under field conditions, was not necessary and was, therefore, not carried out.

B.6.14.1.2 Worker exposure

The highest potential for worker exposure following re-entry will be skin contamination. Risk of inhalation exposure is generally confined to a brief period after application, while the product is drying, which will be rapid under outdoor conditions. In addition, imidacloprid is neither gaseous nor highly volatile (vapour pressure: $4 \cdot 10^{-10}$ Pa, 20 °C). Re-entry is anticipated during the thinning/harvesting of pome fruits and harvesting of tomatoes. The level of exposure will depend largely on the dislodgeable foliar residue (DFR), the length of time the residue remains on the surface of the crops and the degree of contact with the foliage. In the absence of an specific assessment of re-entry exposure the question will be addressed in terms of a "worst-case" scenario, such as that given by Krebs, B. et al. (Uniform principles for safeguarding the health of workers re-entring crop growing areas after application of plant protection products, 1996).

Worker exposure is estimated according to the following formula:

	D	=	DFR x TF x WR x AR x (P)
with	D	=	Dermal Exposure [µg as/person/d]
	DFR	=	Dislodgeable Foliar Residue [µg/cm² per 1 kg as/ha]
	TF	=	Transfer Factor [cm²/person/h]
	WR	=	Work Rate [h/d]
	AR	=	Application Rate of imidacloprid
	P	=	(Protection through clothing if TFs are based on potential exposure)

In a first step the initial DFR (after the spray has dried) can be calculated using a default value of 1 µg as/cm² for an application rate of 1 kg as/ha, according to the following consideration:

1 kg as/ha = 10 µg/cm²;
with two sided leaves ⇨ 5 µg/cm²;
with a LAI (leaf area index) of ca. 3 - 5 ⇨ 1 - 1.66 µg/cm²
resulting in ⇨ ≈ 1 µg as/cm².

This figure was also found as a mean value in available literature and was recently confirmed by Brouwer et al. [Worker exposure to agrochemicals, Ed. R.C. Honeycutt and E.W. Day Jr., pp 119, CRC Press 2001].

Krebs, B. et al. (1996) recommend a transfer factor (TF) of 30.000 cm^2/person x h as an unspecific figure for potential worker exposure in a worst case consideration. The relevant transfer factor, however, is depending on the activity carried out.

In the case of thinning/harvesting of pome fruits a transfer coefficient of 4500 cm²/h has been chosen. In the literature, transfer coefficients for this kind of activity (described as search/reach/pick with primary contact to upper body and hand) range from 4000 cm²/h to 30000 cm²/h [e.g. Krieger et al., Reviews of Environmental Contamination and Toxicology 128, 1 (1992)]. However, it has to be noted that these figures refer to potential transfer coefficients, i.e. exposure estimates derived from these figures describe potential dermal exposure. For further estimation of actual dermal exposure and subsequent systemic exposure both mitigation by work clothing as well as the dermal penetration of (usually) dry foliar residues have to be taken into account.

Generally, potential exposure to harvesters wearing long sleeved shirts and long pants can be assumed to be reduced by 90 % to calculate actual exposure.

Studies measuring exposure of harvesters in apples, peaches and nectarines by passive dosimetry as well as by biomonitoring have shown transfer coefficients for actual dermal exposure ranging from 360 cm²/h to 3600 cm²/h depending on canopy, cultivation and work practices [Formoli and Fong, Cal EPA Worker Health and Safety Branch, HS-1650 (1993)]. A worst case back calculation from urinary metabolites resulted in a transfer coefficient of 4100 cm²/h. Therefore, the chosen transfer coefficient of 4500 cm²/h for actual dermal exposure can be regarded conservative and very much a worst case. This Transfer Factor is also supported by the proposal of the EUROPOEM II Re-entry Working Group. The group recommends an indicative TF value for harvesting fruit trees with bare hands of 4500 (Draft Report of the Re-entry Working Group, Dec. 2002).

Activities performed in fruiting vegetables (tomatoes), e.g. harvesting and pruning, can be grouped to a "reach-and-pick-scenario". According to Krieger et al. [Reviews of Environmental Contamination and Toxicology, Vol. 128 (1992), pp 1] potential dermal Transfer Factors for these activities are in a range of 500 – 8000 cm²/h. EPA uses potential dermal transfer factors in the range of 4000 – 10000 cm²/h or actual factors in the range of 500 – 1500 cm²/h and a high end value of 2500 cm²/h for cucumbers [EPA policy paper, policy number: 003.1]. These factors can be taken for the assessment as there are no big differences in the cultivation of and related work activities in fruiting vegetables between US and Europe. An adequate transfer factor in calculating worker exposure in fruiting tomatoes can be therefore set to 1500 cm²/h.

From all these data and considerations, the dermal exposure of workers can be estimated as follows:

Thinning/harvesting pome fruit

D = DFR x TF x WR x AR
D = 2 (2 applications) x 4500 x 8 x 0.105
D = 7560 µg as/person/d

Taking into account a body weight of 60 kg the dermal exposure amounts to:

D = 0.126 mg as/kg bw/d

Neither further dissipation of residues are considered e.g. during pre harvest interval nor is the presence of dry foliar residues (resulting in less dermal penetration).
Because the dermal absorption rate is assumed to be 100 % (B.6.12) the systemic exposure is identical with the calculated dermal exposure above:

Systemic exposure = 0.126 mg as/kg bw/d.

Fruiting vegetables

D = DFR x TF x WR x AR
D = 2 (2 applications) x 1500 x 8 x 0.150
D = 3600 µg as/person/day

Taking into account a body weight of 60 kg the dermal exposure amounts to:

D = 0.060 mg as/kg bw/day

Neither further dissipation of residues are considered e.g. during pre harvest interval nor the presence of dry foliar residues (resulting in less dermal penetration).
Because the dermal absorption rate is assumed to be 100 % (B.6.12) the systemic exposure is identical with the calculated dermal exposure above:

Systemic exposure = 0.060 mg as/kg bw/day.

Table B.6.14-11: Worker exposure and % of AOEL

Crop	Systemic exposure	% of AOEL (0.15 mg/kg bw/d)
Apple	0.126 mg/kg bw/d	84 %
Tomato	0.060 mg/kg bw/d	40 %

Conclusion:
The comparison of the estimated systemic worker exposure with the systemic AOEL shows that exposure amounts to about 40 - 84 % of the AOEL even with an assumed dermal absorption rate of 100 %. This corresponds to an acceptable level of exposure.
Exposure is calculated for bare hands. Mitigation measures like gloves are frequently used in certain crops to avoid irritation and/or greenish hands especially when working in tomatoes. As these measures have not been considered in the estimate the actual dermal exposure is likely to be even lower.
Based on these estimates it is considered that there is no unacceptable risk for workers re-entering apple orchards and tomato fields following the application of Confidor SL 200.

B.6.14.1.3 Bystander exposure

No official model is available to calculate exposure of bystanders. Therefore, the following definitions and assumptions are considered as described by the notifier:

- A bystander is a person whose presence is quite incidental and unrelated to the work
- Exposure to bystanders can only occur during application via drift
- It is assumed that the bystander will leave the area of a potential exposure after a short period of time (inconvenience through contact with the small droplets of spray drift, through noise of application machinery and eventually through odour)
- It is assumed that machine driven applications in high crops with full foliage represent the worst case for bystander exposure (lost visual range of the tractor driver)
- Repeated exposure is unlikely
- It is assumed that light ordinary clothing is worn; the total uncovered area amounts to 0.4225 m² (i.e. head, back and front of neck, forearms, ½ upper arms and hands: "Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection); Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992").

The application to high crops is regarded to be more critical compared to field crops when assessing the possibility of bystander exposure. Therefore, a calculation is presented only for this scenario.

- Assuming that it takes one minute for the tractor to pass a bystander, the exposure time
  is only the 360th part of the exposure time of the applicator (spraying 6 hours a day)
- Calculation of 100 % deposition (orchard):
  0.105 kg as/ha = 105000 mg as/10 000 m² = 10.5 mg as/m²
- Average spray drift deposition in 7.5 m distance (orchard) = 2.6 %, according to Ganzelmeier et al., Studies on the Spray Drift of Plant Protection Products (Federal Biological Research Centre for Agriculture and Forestry; Berlin; No. 305; 1995)
  Drift data in this publication correspond to multiple exposure events because the sprayer passed the sampling devices several times in various distances. Therefore the spray drift deposition is overestimated for bystander exposure assessment purposes.

Calculation of dermal exposure of a bystander:

D	=	**100 % deposition x drift deposition x exposed area**
D	=	10.5 mg as/m² x 0.026 x 0.4225 m²/person/day
	=	0.115 mg as/person/d
	=	1.9 µg as/kg bw/d (60 kg person)

The inhalation exposure of a bystander is calculated in the same way as for the operator according to the German Model

I	=	**$I^*_{A\ (tractor\ mounted)}$ x WR x AR**
$I^*_{A\ (tractor\ mounted)}$	=	Spec. Exposure Application $_{tractor\ mounted}$ [mg as/person x ha/kg as]
WR	=	Work Rate (orchard): 8 [ha/d = ha/6 h]
AR	=	Application Rate (orchard): 0.105 [kg as/ha]
I	=	0.018 x 8 x 0.105
	=	0.015 mg as/person/d

but adopted to 1 minute (instead of 6 hours for an operator):

I = 0.0311 [mg as/person/d] : 6 [h/d] : 60 [min/h]
= 0.000042 mg as/person/d
= 0.0007 µg as/kg bw/d (60 kg person)

Assessment

Under consideration of the proposed absorption rates (inhalativ 100 %; and also dermal 100 %) the addition of the estimated exposures

D = 1.9 µg as/kg/d and
I = 0.0007 µg as/kg/d

leads to the estimated systemic exposure

Systemic exposure = 1.9 + 0.0007 = 1.9007 µg as/kg/d
= **0.0019 mg/kg bw/d**

Consequently, the amount of the spray dilution which might reach a bystander is far below the proposed systemic AOEL of 0.15 mg/kg bw/d.

B.6.14.2 Gaucho FS 600 Uncoloured

Gaucho FS 600 Uncoloured is a seed dressing liquid containing the insecticidal active substance (as) imidacloprid (600 g/L). It is applied to sugar beet seed at a rate of 90 g as/U, seed rate 1.3 U/ha = 117 g as/ha. The only intended use of Gaucho FS 600 Uncoloured in sugar beet seed treatment is in professional plants.

B.6.14.2.1 Operator exposure

B.6.14.2.1.1 Estimation of operator exposure; risk assessment

Seed treatment

As stated by the notifier the coating of sugar beet seed is a highly technological process which is not performed by agricultural operators but only in closed industrial systems. This industrial process is under control of industrial safety authorities and agricultural professional associations. This guarantees that both the production plants themselves and the way they are run correspond to modern standards, and that safety measures are maintained.

The seeds of sugar and fodder beet are encapsulated in a pill within several steps during the treatment process. This process requires a special coating equipment. The machines are constructed and designed for the special purpose of seed treatment which is mostly performed under negative pressure. With respect to operator safety these plants do fulfil highest safety standards which are permanently under progress. The whole treatment system is connected to an air filtering system. In addition, regularly cleaning intervals are advised to avoid dispersion of dust. Operators of these plants are trained in the activities they have to perform during the treatment process. They wear protective clothing (e.g. overalls), gloves, goggles and respiratory protection according to good occupational practice.

Sowing

As stated by the notifier the sugar beet seeds dressed with the active substance imidacloprid are covered by a protective coating which is highly resistant against mechanical abrasion. Operator exposure via the dermal or inhalation route during loading and sowing of the treated

seed is therefore unlikely. However, according to good agricultural practice it is recommended that operators should wear adequate clothing during loading/sowing of treated seed (e.g. long sleeved shirt, long trousers, gloves when handling treated seed or contaminated surfaces).

Assessment
As stated by the notifier during the sugar/fodder beet seed treatment process operator exposure is regarded to be very low due to the very high safety level (technical equipment, training of the operators, protective clothing) of the professional plants. In addition, operator exposure is unlikely during loading/sowing of the treated seed because the active substance is covered by a protective coating which is highly resistant against mechanical abrasion.
Therefore, an unacceptable risk for the operator wearing adequate clothing is not anticipated both during seed treatment and loading/sowing of the treated sugar beet seed.

B.6.14.2.1.2 Measurement of operator exposure – seed treatment

Measurement of operator exposure was not performed because estimates of exposure to imidacloprid do not reveal unacceptable risks to agricultural workers.

Appendix 1

German model: Operator exposure for imidacloprid in apples - air blast sprayer

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	SL	$D_{M(H)}$	=	2.4 mg/person x kg as
Application technique:	High crops: tractor-mounted	$D_{A(H)}$	=	0.7 mg/person x kg as
Application rate:	0.105 kg imidacloprid /ha	$D_{A(B)}$	=	9.6 mg/person x kg as
Area treated per day:	8 ha	$D_{A(C)}$	=	1.2 mg/person x kg as
		I_M	=	0.0006 mg/person x kg as
		I_A	=	0.018 mg/person x kg as

Route of exposure			**Scenario 1** (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.; garment: appl.)
Dermal/mixing					
exposure (hands):	$D_{M(H)}$	=	2.4 x 0.105 x 8		
		=	2.016 mg/person	0.0202 mg/person*1	0.0202 mg/person*1
Dermal/application					
exposure (hands, body, head)	$D_{A(H)}$	=	0.7 x 0.105 x 8		
		=	0.588 mg/person	0.588 mg/person	0.00588 mg/person*1
	$D_{A(B)}$	=	9.6 x 0.105 x 8		
		=	8.064 mg/person	8.064 mg/person	0.4032 mg/person*2
	$D_{A(C)}$	=	1.2 x 0.105 x 8		
		=	1.008 mg/person	1.008 mg/person	1.008 mg/person
Total dermal exposure		=	**11.676 mg/person**	**9.680 mg/person**	**1.437 mg/person**
Inhalation/mixing					
	I_M	=	0.0006 x 0.105 x 8		
		=	0.0005 mg/person	0.0005 mg/person	0.0005 mg/person
Inhalation/application					
	I_A	=	0.018 x 0.105 x 8		
		=	0.01512 mg/person	0.01512 mg/person	0.01512 mg/person
Total inhalation exposure		=	**0.01562 mg/pers.**	**0.01562 mg/pers.**	**0.01562 mg/pers.**

*1 reduction factor of gloves = 0.01
*2 reduction factor of protective clothing = 0.05

Appendix 2

German model: Operator exposure for imidacloprid in apples - hand held sprayer

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	SL	$\mathbf{D_{M(H)}}$	=	205 mg/person x kg as
Application technique:	High crops: hand held	$\mathbf{D_{A(H)}}$	=	10.6 mg/person x kg as
Application rate:	0.105 kg imidacloprid /ha	$\mathbf{D_{A(B)}}$	=	25 mg/person x kg as
Area treated per day:	1 ha	$\mathbf{D_{A(C)}}$	=	4.8 mg/person x kg as
		$\mathbf{I_M}$	=	0.05 mg/person x kg as
		$\mathbf{I_A}$	=	0.3 mg/person x kg as

Route of exposure			**Scenario 1** (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.; garment: appl.)
Dermal/mixing					
exposure (hands):	$\mathbf{D_{M(H)}}$	=	205 x 0.105 x 1		
		=	21.525 mg/person	0.21525 mg/pers.*1	0.21525 mg/pers.*1
Dermal/application					
exposure (hands, body, head)	$\mathbf{D_{A(H)}}$	=	10.6 x 0.105 x 1		
		=	1.113 mg/person	1.113 mg/person	0.001113 mg/pers.*1
	$\mathbf{D_{A(B)}}$	=	25 x 0.105 x 1		
		=	2.625 mg/person	2.625 mg/person	0.13125 mg/person*2
	$\mathbf{D_{A(C)}}$	=	4.8 x 0.105 x 1		
		=	0.504 mg/person	0.504 mg/person	0.504 mg/person
Total dermal exposure		=	**25.767 mg/person**	**4.457 mg/person**	**0.862 mg/person**
Inhalation/mixing					
	$\mathbf{I_M}$	=	0.05 x 0.105 x 1		
		=	0.00525 mg/person	0.00525 mg/person	0.00525 mg/person
Inhalation/application					
	$\mathbf{I_A}$	=	0.3 x 0.105 x 1		
		=	0.0315 mg/person	0.0315 mg/person	0.0315 mg/person
Total inhalation exposure		=	**0.03675 mg/pers.**	**0.03675 mg/pers.**	**0.03675 mg/pers.**

*1 reduction factor of gloves = 0.01
*2 reduction factor of protective clothing = 0.05

Appendix 3

German model: Operator exposure for imidacloprid in tomatoes, tractor boom sprayer

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	SL	$D_{M(H)}$	=	2.4 mg/person x kg as
Application technique:	Field crops: tractor-mounted	$D_{A(H)}$	=	0.38 mg/person x kg as
Application rate:	0.1 kg imidacloprid /ha	$D_{A(B)}$	=	1.6 mg/person x kg as
Area treated per day:	20 ha	$D_{A(C)}$	=	0.06 mg/person x kg as
		I_M	=	0.0006 mg/person x kg as
		I_A	=	0.001 mg/person x kg as

Route of exposure			**Scenario 1** (no PPE)	**Scenario 2** (gloves: m/l)	**Scenario 3** (gloves: m/l and appl.; garment: appl.)
Dermal/mixing					
exposure (hands):	$D_{M(H)}$	=	2.4 x 0.1 x 20		
		=	4.8 mg/person	0.048 mg/person*1	0.048 mg/person*1
Dermal/application					
exposure (hands, body, head)	$D_{A(H)}$	=	0.38 x 0.1 x 20		
		=	0.76 mg/person	0.76 mg/person	0.0076 mg/person
	$D_{A(B)}$	=	1.6 x 0.1 x 20		
		=	3.2 mg/person	3.2 mg/person	0.16 mg/person*2
	$D_{A(C)}$	=	0.06 x 0.1 x 20		
		=	0.12 mg/person	0.12 mg/person	0.12 mg/person
Total dermal exposure		=	**8.880 mg/person**	**4.128 mg/person**	**0.336 mg/person**
Inhalation/mixing					
	I_M	=	0.0006 x 0.1 x 20		
		=	0.0012 mg/person	0.0012 mg/person	0.0012 mg/person
Inhalation/application					
	I_A	=	0.001 0.1 x 20		
		=	0.002 mg/person	0.002 mg/person	0.0012 mg/person
Total inhalation exposure		=	**0.0032 mg/person**	**0.0032 mg/person**	**0.0032 mg/person**

*1 reduction factor of gloves = 0.01

*2 reduction factor of protective clothing = 0.05

Appendix 4

UK POEM: Operator exposure for imidacloprid in apples - air blast sprayer; no PPE

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.07	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.53	litres product/ha		
Work rate	15	ha/d		
Number of operations	2	per day		
Hand contamination	0.02	mL/d		
Protective clothing	none			
Transmission to skin	100	%		
Dermal exposure to formulation	0.02	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1500	litres spray/ha		
Volume of surface contamination	400	mL/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	none	permeable	permeable	
Penetration	100	2	5	%
Dermal exposure	10	5.2	5	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.02	mL/d	121.2	mL/d
Concentration of as	200	mg/mL	0.07	mg/mL
Dermal exposure to as	4	mg/d	8.484	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	4	mg/d	8.484	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	mL/h		
Duration of exposure	6	h		
Concentration of as	0.07	mg/mL		
Inhalation exposure to as	0.021	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.021	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	12.505	mg/d		
Operator body weight	60	kg		
Operator exposure	0.2084	mg/kg bw/d		

Appendix 5

UK POEM: Operator exposure for imidacloprid in apples - air blast sprayer; gloves during mixing/loading

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.07	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.53	litres product/ha		
Work rate	15	ha/d		
Number of operations	2	per day		
Hand contamination	0.02	mL/d		
Protective clothing	gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.001	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1500	litres spray/ha		
Volume of surface contamination	400	mL/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	none	permeable	permeable	
Penetration	100	2	5	%
Dermal exposure	10	5.2	5	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	121.2	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.001	mL/d	121.2	mL/d
Concentration of as	200	mg/mL	0.07	mg/mL
Dermal exposure to as	0.2	mg/d	8.484	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	0.2	mg/d	8.484	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	mL/h		
Duration of exposure	6	h		
Concentration of as	0.07	mg/mL		
Inhalation exposure to as	0.021	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.021	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	8.705	mg/d		
Operator body weight	60	kg		
Operator exposure	0.1451	mg/kg bw/d		

Appendix 6

UK POEM: Operator exposure for imidacloprid in apples - air blast sprayer; gloves during mixing/loading and application

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.07	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.53	litres product/ha		
Work rate	15	ha/d		
Number of operations	2	per day		
Hand contamination	0.02	mL/d		
Protective clothing	gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.001	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1500	litres spray/ha		
Volume of surface contamination	400	mL/h		
Distribution	Hands	Trunk	Legs	
	10	65	25	%
Clothing	gloves	permeable	permeable	
Penetration	10	2	5	%
Dermal exposure	4	5.2	5	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	85.2	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.001	mL/d	85.2	mL/d
Concentration of as	200	mg/mL	0.07	mg/mL
Dermal exposure to as	0.2	mg/d	5.964	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	0.2	mg/d	5.964	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.05	mL/h		
Duration of exposure	6	h		
Concentration of as	0.07	mg/mL		
Inhalation exposure to as	0.021	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.021	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	6.185	mg/d		
Operator body weight	60	kg		
Operator exposure	0.1031	mg/kg bw/d		

Appendix 7

UK POEM: Operator exposure for imidacloprid in tomatoes - tractor boom sprayer with hydraulic nozzles; no PPE

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.1	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.5	litres product/ha		
Work rate	30	ha/d		
Number of operations	3	per day		
Hand contamination	0.03	mL/d		
Protective clothing	none			
Transmission to skin	100	%		
Dermal exposure to formulation	0.03	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1000	litres spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	none	permeable	permeable	
Penetration	100	5	15	%
Dermal exposure	6.5	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.03	mL/d	41.55	mL/d
Concentration of as	200	mg/mL	0.1	mg/mL
Dermal exposure to as	6	mg/d	4.155	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	6	mg/d	4.155	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of as	0.1	mg/mL		
Inhalation exposure to as	0.006	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.006	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	10.161	mg/d		
Operator body weight	60	kg		
Operator exposure	0.1694	mg/kg bw/d		

Appendix 8

UK POEM: Operator exposure for imidacloprid in tomatoes - tractor boom sprayer with hydraulic nozzles; gloves during mixing/loading

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.1	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.5	litres product/ha		
Work rate	30	ha/d		
Number of operations	3	per day		
Hand contamination	0.03	mL/d		
Protective clothing	gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.0015	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1000	litres spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	none	permeable	permeable	
Penetration	100	5	15	%
Dermal exposure	6.5	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	41.55	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.0015	mL/d	41.55	mL/d
Concentration of as	200	mg/mL	0.1	mg/mL
Dermal exposure to as	0.3	mg/d	4.155	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	0.3	mg/d	4.155	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of as	0.1	mg/mL		
Inhalation exposure to as	0.006	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.006	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	0.951	mg/d		
Operator body weight	60	kg		
Operator exposure	0.01585	mg/kg bw/d		

Appendix 9

UK POEM: Operator exposure for imidacloprid in tomatoes - tractor boom sprayer with hydraulic nozzles; gloves during mixing/loading and application

PRODUCT DATA				
Product	Confidor SL 200			
Active substance	Imidacloprid			
Concentration	200	mg/mL		
Formulation type	SL			
Maximum in-use as concentration	0.1	mg/mL		
EXPOSURE DURING MIXING AND LOADING				
Container size	5	litres		
Hand contamination/operation	0.01	mL		
Application dose	0.5	litres product/ha		
Work rate	30	ha/d		
Number of operations	3	per day		
Hand contamination	0.03	mL/d		
Protective clothing	gloves			
Transmission to skin	5	%		
Dermal exposure to formulation	0.0015	mL/d		
EXPOSURE DURING SPRAY APPLICATION				
Application volume	1000	litres spray/ha		
Volume of surface contamination	10	mL/h		
Distribution	Hands	Trunk	Legs	
	65	10	25	%
Clothing	gloves	permeable	permeable	
Penetration	10	5	15	%
Dermal exposure	0.65	0.05	0.375	mL/h
Duration of exposure	6	h		
Total dermal exposure to spray	6.45	mL/d		
ABSORBED DOSE				
	Mix/load		Application	
Dermal exposure	0.0015	mL/d	6.45	mL/d
Concentration of as	200	mg/mL	0.1	mg/mL
Dermal exposure to as	0.3	mg/d	0.645	mg/d
Percent absorbed	100	%	100	%
Absorbed dose	0.3	mg/d	0.645	mg/d
INHALATION EXPOSURE DURING SPRAYING				
Inhalation exposure	0.01	mL/h		
Duration of exposure	6	h		
Concentration of as	0.1	mg/mL		
Inhalation exposure to as	0.006	mg/d		
Percent absorbed	100	%		
Absorbed dose	0.006	mg/d		
PREDICTED EXPOSURE				
Total absorbed dose	4.461	mg/d		
Operator body weight	60	kg		
Operator exposure	0.0744	mg/kg bw/d		

Appendix 10

Operator exposure for imidacloprid in tomatoes - greenhouses: knapsack sprayer
Mixing/loading: German model – as for hand held spraying
Application: greenhouse data – ECON, 1996

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	SL	$\mathbf{D_{M(H)}}$	=	205 mg/person x kg as.
Application technique:	knapsack sprayer	**with PPE**		2.05 mg/person x kg as.
Application rate:	0.15 kg imidacloprid /ha	$\mathbf{D_{A(H)}}$	=	13.2 mg/person x kg as
Area treated per day:	1 ha	**with PPE**		0.008 mg/person x kg as
		$\mathbf{D_{A(B)}}$	=	82.5 mg/person x kg as
		with PPE		0.228 mg/person x kg as
		$\mathbf{D_{A(C)}}$	=	1.56 mg/person x kg as
		$\mathbf{I_M}$	=	0.05 mg/person x kg as
		$\mathbf{I_A}$	=	0.108 mg/person x kg as

Route of exposure			Scenario 1 (no PPE)	Scenario 2 (with PPE)
Dermal/mixing				
exposure (hands):	$\mathbf{D_{M(H)}}$	=	205 x 0.15 x 1	2.05 x 0.15 x 1
		=	30.75 mg/person	0.3075 mg/person
Dermal/application				
exposure (hands, body, head)	$\mathbf{D_{A(H)}}$	=	13.2 x 0.15 x 1	0.008 x 0.15 x 1
		=	1.98 mg/person	0.0012 mg/person
	$\mathbf{D_{A(B)}}$	=	82.5 x 0.15 x 1	0.228 x 0.15 x 1
		=	12.375 mg/person	0.0342 mg/person
	$\mathbf{D_{A(C)}}$	=	1.56 x 0.15 x 1	1.56 x 0.15 x 1
		=	0.234 mg/person	0.234 mg/person
Total dermal exposure		=	**45.339 mg/person**	**0.5769 mg/person**
Inhalation/mixing				
	$\mathbf{I_M}$	=	0.05 x 0.15 x 1	0.05 x 0.15 x 1
		=	0.0075 mg/person	0.0075 mg/person
Inhalation/application				
	$\mathbf{I_A}$	=	0.108 x 0.15 x 1	0.108 x 0.15 x 1
		=	0.0162 mg/person	0.0162 mg/person
Total inhalation exposure		=	**0.0237 mg/person**	**0.0237 mg/person**

Appendix 11

Operator exposure for imidacloprid in tomatoes - greenhouses: Hand held spraying with spray lance connected to stationary large tank
Mixing/loading: German model – as for tractor mounted equipment
Application: greenhouse data – ECON, 1996

Assumptions and input parameters considered for the estimation of the operator exposure:

Formulation type:	SL	$D_{M(H)}$	= 2.4 mg/person x kg as.
Application technique:	spraying with lance	**with PPE**	0.024 mg/person x kg as.
Application rate:	0.15 kg imidacloprid /ha	$D_{A(H)}$	= 13.2 mg/person x kg as
Area treated per day:	1 ha	**with PPE**	0.008 mg/person x kg as
		$D_{A(B)}$	= 82.5 mg/person x kg as
		with PPE	0.228 mg/person x kg as
		$D_{A(C)}$	= 1.56 mg/person x kg as
		I_M	= 0.0006 mg/person x kg as
		I_A	= 0.108 mg/person x kg as

Route of exposure			Scenario 1 (no PPE)	Scenario 2 (with PPE)
Dermal/mixing				
exposure (hands):	$D_{M(H)}$	=	2.4 x 0.15 x 1	0.024 x 0.15 x 1
		=	0.36 mg/person	0.0036 mg/person
Dermal/application				
exposure (hands, body, head)	$D_{A(H)}$	=	13.2 x 0.15 x 1	0.008 x 0.15 x 1
		=	1.98 mg/person	0.0012 mg/person
	$D_{A(B)}$	=	82.5 x 0.15 x 1	0.228 x 0.15 x 1
		=	12.375 mg/person	0.0342 mg/person
	$D_{A(C)}$	=	1.56 x 0.15 x 1	1.56 x 0.15 x 1
		=	0.234 mg/person	0.234 mg/person
Total dermal exposure		**=**	**14.949 mg/person**	**0.273 mg/person**
Inhalation/mixing				
	I_M	=	0.0006 x 0.15 x 1	0.0006 x 0.15 x 1
		=	0.00009 mg/person	0.00009 mg/person
Inhalation/application				
	I_A	=	0.108 x 0.15 x 1	0.108 x 0.15 x 1
		=	0.0162 mg/person	0.0162 mg/person
Total inhalation exposure		**=**	**0.01629 mg/person**	**0.01629 mg/person**

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.1; AIIA-6.2	Karl, W. and Klein, O.	1992	(Pyridinyl-^{14}C-methylene)-imidacloprid: Distribution of the metabolites in some organs at different times following single oral administration to rats. PF3635 GLP, unpublished RIP2003-1657	Y	BAY
AIIA-5.1; AIIA-6.2	Karl, W. and Klein, O.	1992	(Pyridinyl-^{14}C-methylene)-imidacloprid: Distribution of the metabolites in some organs at different times following single oral administration to rats. PF3635 ! M 1820216-0 GLP, unpublished TOX2003-1970	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O.	1987	[^{14}C]-NTN 33893: Investigations on the distribution of the total radioactivity in the rat by whole-body autoradiography. PF2891 GLP, unpublished RIP2003-1653	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O.	1990	Imidacloprid - WAK 3839: Comparison of biokinetic behaviour and metabolism in the rat following single oral dosage and investigation of the metabolism after chronic feeding of imidacloprid to rats and mice. PF3432 GLP, unpublished RIP2003-1655	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O.	1987	(^{14}C)-NTN 33893: Biokinetic part of the 'General metabolism study' in the rat. PF2889 GLP, unpublished TOX2003-1965	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O.	1987	[^{14}C]-NTN 33893: Investigations on the distribution of the total radioactivity in the rat by whole-body autoradiography. PF2891 GLP, unpublished TOX2003-1966	Y	BAY

[1] Only notifier listed

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.1; AIIA-6.2	Klein, O.	1987	(^{14}C)-NTN 33893: Biokinetic part of the 'General metabolism study' in the rat. PF2889 GLP, unpublished RIP2003-1652	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O.	1990	Imidacloprid: WAK 3839: Comparison of biokinetic behaviour and metabolism in the rat following single oral dosage and investigation of the metabolism after chronic feeding of imidacloprid to rats and mice. PF3432 ! M 71810016 GLP, unpublished TOX2003-1968	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O. and Brauner, A.	1991	Imidazolidine-4,5-[^{14}C]-imidacloprid: Investigation of the biokinetic behaviour and metabolism in the rat. PF3629 GLP, unpublished TOX2003-1967	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O. and Brauner, A.	1991	Imidazolidine-4,5-[^{14}C]-imidacloprid: Investigation of the biokinetic behaviour and metabolism in the rat. PF3629 GLP, unpublished RIP2003-1654	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O. and Karl, W.	1990	Methylene-(^{14}C)-imidacloprid: Metabolism part of the general metabolism study in the rat. PF3316 GLP, unpublished RIP2003-1656	Y	BAY
AIIA-5.1; AIIA-6.2	Klein, O. and Karl, W.	1990	Methylene-(^{14}C)-imidacloprid: Metabolism part of the general metabolism study in the rat. PF3316 ! M 1820176-5 GLP, unpublished TOX2003-1969	Y	BAY
AIIA-5.2	Kroetlinger, F.	1990	NTN 33893 (c.n. imidacloprid (proposed): Study for acute intraperitoneal toxicity in rats. 19245 ! T 2033141 GLP, unpublished TOX2003-1977	Y	BAY
AIIA-5.2.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893 - Study for acute oral toxicity to rats. 18594 ! MO-00-003582 GLP, unpublished AVS2003-302	Y	BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.2.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893 - Study for acute oral toxicity to mice. 18593 ! MO-99-002827 GLP, unpublished AVS2003-300	Y	BAY
AIIA-5.2.1	Bomann, W.	1991	NTN 33893 CNS (c.n.: Imidacloprid (vorgeschl.)): Untersuchungen zur akuten oralen Toxizität an Ratten. 20637 GLP, unpublished TOX1999-1070	Y	BAY
AIIA-5.2.1	Bomann, W.	1991	NTN 33893 AMP (proposed c.n.: Imidacloprid): Study for acute oral toxicity to rats. 20591 ! T 8038043 GLP, unpublished TOX2003-1971	Y	BAY
AIIA-5.2.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893: Untersuchungen zur akuten oralen Toxizität an Maeusen. 18593 GLP, unpublished TOX1999-1071	Y	BAY
AIIA-5.2.1; AIIIA-7.1.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893: Study for acute oral toxicity to rats. 18594 GLP, unpublished TOX2004-94	Y	BAY
AIIA-5.2.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893: Study for acute oral toxicity to mice. 18593 ! T 4033062 GLP, unpublished TOX2004-96	Y	BAY
AIIA-5.2.1	Bomann, W.	1991	NTN 33893 CNS (c.n.: Imidacloprid (proposed.): Study for acute oral toxicity in rats. 20637 T 7039564 GLP, unpublished TOX2004-95	Y	BAY
AIIA-5.2.1; AIIIA-10.3	Bomann, W.	1989	NTN 33893: Untersuchungen zur akuten oralen Toxizitaet an Ratten. 18594 GLP, unpublished TOX1999-1069	Y	BAY
AIIA-5.2.1; AIIA-5.8.2	Kroetlinger, F.	1994	NTN 33893 (c.n.: Imidacloprid [proposed]) FCR 1272 (c.n.: Cyfluthrin): Study for combination toxicity in rats. 23420 ! T 8055008 ! T 7055007 ! T 0055109 GLP, unpublished TOX2001-1764	Y	BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.2.2; AIIIA-7.1.2	Kroetlinger, F.	1989	NTN 33893 (c.n. imidacloprid (proposed): Study for acute dermal toxicity to rats. 18532 ! T 5033063 GLP, unpublished TOX2003-1972	Y	BAY
AIIA-5.2.3; AIIA-5.3.3	Pauluhn, J.	1988	NTN 33893: Study for acute inhalation toxicity in the rat in accordance with OECD guideline no. 403. 16777 ! T 2025951 GLP, unpublished TOX2003-1973	Y	BAY
AIIA-5.2.4; AIIIA-7.1.4	Pauluhn, J.	1988	NTN 33893: Study for irritant/corrosive potential on the skin (rabbit) according to OECD guideline no. 404. 16455 ! T 8025515 GLP, unpublished TOX2003-1974	Y	BAY
AIIA-5.2.5; AIIIA-7.1.5	Pauluhn, J.	1988	NTN 33893: Study for irritant/corrosive potential on the eye (rabbit) according to OECD guideline no. 405. 16456 ! T 8025515 GLP, unpublished TOX2003-1975	Y	BAY
AIIA-5.2.6	Ohta, K.	1988	NTN 33893 technical: Study for skin sensitising effect on guinea pigs (maximisation test). 16533 ! T 9025651 GLP, unpublished TOX2003-1976	Y	BAY
AIIA-5.3.1	Bloch, I., Frei, T., Luetkemeier, H., Vogel, W. and Wilson, J.	1987	28-day oral range-finding toxicity (feeding) study with NTN 33893 tech. in the dog. R4196 not GLP, unpublished TOX2003-1978	Y	BAY
AIIA-5.3.2	Eiben, R. and Hartmann, E.	1988	NTN 33893 - Pilot range-finding study for a cancerogenesis study on B6C3F1 mice (one hundred seven day feeding study). 17280 ! T 7024524 not GLP, unpublished TOX2003-1981	Y	BAY
AIIA-5.3.2	Eiben, R. and Rinke, M.	1989	NTN 33893: Subchronic toxicity study on wistar rats (administration in the feed for 96 days). 18187 ! T 7027297 GLP, unpublished TOX2003-1980	Y	BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.3.2	Eiben, R. and Sander, E.	1988	NTN 33893: Pilot range-finding study for a chronic toxicity study on Wistar rats (ninety-eight day feeding study). 17279 ! T 7024489 not GLP, unpublished TOX2003-1979	Y	BAY
AIIA-5.3.2	Ruf, J. and Sander, E.	1990	NTN 33893 technical - Subchronic toxicity study on dogs in oral administration (thirteen-week feeding study). 18732 ! T 3025763 GLP, unpublished TOX2003-1984	Y	BAY
AIIA-5.3.3	Flucke, W.	1990	NTN 33893 techn. - Study for subacute dermal toxicity in the rabbit. 19152 ! T 7029592 GLP, unpublished TOX2003-1986	Y	BAY
AIIA-5.3.3	Pauluhn, J.	1989	NTN 33893 (proposed common name: Imidacloprid) - Subacute inhalation toxicity study on the rat according to OECD guideline no. 412. 18199 ! 3027635 GLP, unpublished TOX2003-1985	Y	BAY
AIIA-5.4.1	Cifone, M.A.	1988	Mutagenicity test on NTN 33893 in the rat primary hepatocyte unscheduled DNA synthesis assay. R4631 GLP, unpublished TOX2003-1994	Y	BAY
AIIA-5.4.1; AIIA-5.8.1	Fautz, R.	1989	Unscheduled DNA synthesis in primary hepatocytes of male rats *in vitro* with WAK 3839. R4746 GLP, unpublished TOX2003-2023	Y	BAY
AIIA-5.4.1	Herbold, B.A.	1992	NTN 33893 AMP W - Salmonella/microsome test. 21775 GLP, unpublished TOX2003-1989	Y	BAY
AIIA-5.4.1	Herbold, B.A.	1988	NTN 33893 - Test on *S. cerevisiae* D7 to evaluate for induction of mitotic recombination. 16832 GLP, unpublished TOX2003-1993	Y	BAY

Annex point/ reference number	Author(s)	Year	Title source (where different from company) report no. GLP or GEP status (where relevant), published or not BBA registration number	Data protection claimed Y/N	Owner[1]
AIIA-5.4.1	Herbold, B.A.	1989	NTN 33893 - *In vitro* cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. 18092 + Addendum 18092A, date 24.08.1989 GLP, unpublished TOX2003-1997	Y	BAY
AIIA-5.4.1	Herbold, B.A.	1991	NTN 33893 AMP - Salmonella/microsome test. 20090 GLP, unpublished TOX2003-1988	Y	BAY
AIIA-5.4.1	Herbold, B.A.	1989	NTN 33893 - Salmonella/microsome test to evaluate for point mutagenic effects. 17577 GLP, unpublished TOX2003-1987	Y	BAY
AIIA-5.4.1	Lehn, H.	1989	NTN 33893 - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay *in vitro*. 17578 GLP, unpublished TOX2003-1992	Y	BAY
AIIA-5.4.1	Putman, D.L. and Morris, M.J.	1989	BAY NTN 33893 - Sister chromatid exchange assay in chinese hamster ovary cells. BC1149 GLP, unpublished TOX2003-1996	Y	BAY
AIIA-5.4.1	Taalman, M.	1988	Clastogenic evaluation of NTN 33893 in an *in vitro* cytogenetic assay measuring sister chromatid exchange in chinese hamster ovary (CHO) cells. R4407 GLP, unpublished TOX2003-1995	Y	BAY
AIIA-5.4.1	Watanabe, M.	1990	NTN 33893 - Rec-assay with spores in the bacterial System. RA90016 GLP, unpublished TOX2003-1991	Y	BAY
AIIA-5.4.1	Watanabe, M.	1991	NTN 33893 - Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*). RA91002 GLP, unpublished TOX2003-1990	Y	BAY

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AIIA-5.4.1; AIIA-5.8.1	Fautz, R.	1989	Unscheduled DNA synthesis in primary hepatocytes of male rats *in vitro* with WAK 3839. R4746 GLP, unpublished TOX2003-2023	Y	BAY
AIIA-5.4.2	Herbold, B.A.	1993	NTN 33893 - Sister chromatid exchange in bone marrow of chinese hamsters *in vivo*. 18093 GLP, unpublished TOX2003-2000	Y	BAY
AIIA-5.4.2	Herbold, B.A.	1988	NTN 33893 - Micronucleus-test on the mouse to evaluate for clastogenic effects. 16837 GLP, unpublished TOX2003-1999	Y	BAY
AIIA-5.4.2	Herbold, B.A.	1989	NTN 33893 - *In vivo* cytogenetic study of the bone marrow in chinese hamster to evaluate for induced clastogenic effects. 18557 GLP, unpublished TOX2003-1998	Y	BAY
AIIA-5.4.3	Voelkner, W.	1990	Mouse germ-cell cytogenetic assay with NTN 33893. R5063 GLP, unpublished TOX2003-2001	Y	BAY
AIIA-5.5	Allen, T.R., Frei, T., Luetkemeier, H., Vogel, O., Biedermann, K. and Wilson, J.	1992	52-week oral toxicity (feeding) study with NTN 33893 technical in the dog. R4856 GLP, unpublished TOX2003-2004	Y	BAY
AIIA-5.5	Eiben, R.	1991	NTN 33893 (proposed common name: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months) - supplementary MTD study for two-year study T1025699. 20541 ! T 3030055 GLP, unpublished TOX2003-1983	Y	BAY
AIIA-5.5	Eiben, R. and Kaliner, G.	1991	NTN 33893 (proposed c.n.: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months). 19925 ! T 1025699 GLP, unpublished TOX2003-1982	Y	BAY

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AIIA-5.5	Watta-Gebert, B.	1991	NTN 33893 (proposed common name: Imidacloprid) - Carcinogenicity study in B6C3F1 mice (supplementary MTD testing for study T5025710 with administration in diet over a 24-month period). 20769 ! T 4029986 GLP, unpublished TOX2003-2003	Y	BAY
AIIA-5.5	Watta-Gebert, B. and Rehkemper, U.	1991	NTN 33893 (proposed common name Imidacloprid) - Carcinogenicity study on B6C3F1 mice (administration in the food for 24 months). 19931 ! T 5025710 GLP, unpublished TOX2003-2002	Y	BAY
AIIA-5.6.1; AIIIA-10.3	Suter, P., Biedermann, K., Luetkemeier, H., Wilson, J.T. and Terrier, C.	1992	Multiple generation reproduction study with NTN 33893 technical in rats. R5097 GLP, unpublished TOX2003-2006	Y	BAY
AIIA-5.6.1; AIIIA-10.3	Suter, P., Biedermann, K., Luetkemeier, H., Wilson, J.T. and Terrier, C.	1992	Multiple generation reproduction study with NTN 33893 technical in rats. R5097 ! MO-00-004229 GLP, unpublished AVS2003-303	Y	BAY
AIIA-5.6.1	Suter, P., Vogel, W., Wilson, J.T. and Terrier, C.	1990	NTN 33893 technical - Range finding study to multiple generation study in the rat. R4955 ! T 6025162 ! 087052 GLP, unpublished TOX2003-2005	Y	BAY
AIIA-5.6.2	Becker, H., Vogel, W. and Terrier, C.	1988	Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rabbit. R4583 GLP, unpublished TOX2003-2008	Y	BAY
AIIA-5.6.2	Becker, H., Vogel, W. and Terrier, C.	1988	Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rat. R4582 GLP, unpublished TOX2003-2007	Y	BAY
AIIA-5.8.1	Heidemann, A.	1989	Chromosome aberration assay in chinese hamster V79 cells *in vitro* with WAK 3839. R4849 GLP, unpublished TOX2003-2024	Y	BAY

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AIIA-5.8.1	Herbold, B.A.	1989	WAK 3839 or NTN 37571 - Micronucleus test on the mouse after intraperitoneal injection. 18407 GLP, unpublished TOX2003-2029	Y	BAY
AIIA-5.8.1	Herbold, B.A.	1989	WAK 3839 - Micronucleus test on the mouse after oral application. 18406 GLP, unpublished TOX2003-2028	Y	BAY
AIIA-5.8.1	Kroetlinger, F. and Newman, A.J.	1992	WAK 3839: Subchronic toxicological study on rats (Twelve-week administration on drinking water). 21140 ! T 5033324 ! MO-00-006064 GLP, unpublished TOX2003-2030	Y	BAY
AIIA-5.8.1	Lehn, H.	1989	WAK 3839 - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay *in vitro*. 18281 GLP, unpublished TOX2003-2022	Y	BAY
AIIA-5.8.1	Lehn, H.	1989	WAK 3839 - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay *in vitro*. 17757 GLP, unpublished TOX2003-2021	Y	BAY
AIIA-5.8.1	Nakazato, Y.	1993	NTN 38014 - Acute oral toxicity study on rats. RA91018 ! 90A050 GLP, unpublished TOX2003-2020	Y	BAY
AIIA-5.8.1	Nakazato, Y.	1990	NTN 37571 - Acute toxicity study on mice. RS88038 not GLP, unpublished TOX2003-2019	Y	BAY
AIIA-5.8.1	Nakazato, Y.	1990	NTN 37571 - Oral acute toxicity study on rats. RS89007 not GLP, unpublished TOX2003-2018	Y	BAY
AIIA-5.8.1	Ohta, K.	1993	NTN 35884 - Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*). RA91040 ! 91A030 GLP, unpublished TOX2003-2011	Y	BAY

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AIIA-5.8.1	Ohta, K.	1991	WAK 3839 - Acute oral toxicity study on rats. RA91017 GLP, unpublished TOX2003-2012	Y	BAY
AIIA-5.8.1	Ohta, K.	1993	NTN 35884 - Acute oral toxicity study on rats. RA91039 ! 91A027 GLP, unpublished TOX2003-2010	Y	BAY
AIIA-5.8.1	Ohta, K.	1991	NTN 33519 - Acute oral toxicity study on rats. RA91023 ! 91A005 GLP, unpublished TOX2003-2009	Y	BAY
AIIA-5.8.1	Usami, M.	1988	NTN 37571 - Micronucleus test on the mice after i.p. treatment - pilot study. RS88041 not GLP, unpublished TOX2003-2027	Y	BAY
AIIA-5.8.1	Usami, M.	1989	NTN 37571 - *In vitro* cytogenetic assay measuring chromosome aberrations in CHO-K1 cells - A pilot study. RP88008 not GLP, unpublished TOX2003-2025	Y	BAY
AIIA-5.8.1	Watanabe, M.	1991	WAK 3839 - Rec-assay with spores in the bacterial system. RA91015 ! 91A001 GLP, unpublished TOX2003-2014	Y	BAY
AIIA-5.8.1	Watanabe, M.	1993	NTN 38014 - Reverse mutation assay (*Salmonella typhimurium* and *Escherichia coli*). RA91019 ! 91A002 GLP, unpublished TOX2003-2016	Y	BAY
AIIA-5.8.1	Watanabe, M.	1990	WAK 3839 - Reverse mutation assay (*Salmonella tyhimurium* and *Escherichia coli*). RA90035 GLP, unpublished TOX2003-2015	Y	BAY
AIIA-5.8.1	Watanabe, M.	1991	NTN 33519 - Reverse mutation assay (*Salmonella typhimurium* and *Eschericha coli*). RA91024 ! 91A006 GLP, unpublished TOX2003-2013	Y	BAY

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AIIA-5.8.2	Andrews, P.	2002	Flumethrin & Imidacloprid (c.n.: Flumethrin & Imidacloprid) - Study for acute oral combination toxicity in rats. 31644 ! T 7068139 GLP, unpublished TOX2003-2035	Y	BAY
AIIA-5.8.2	Kroetlinger, F.	1994	NTN 33893 (c.n. imidacloprid [proposed]); SRA 5172 (c.n. methamidophos) - Study for combination toxicity in rats. 23454 ! T 8055008 + Addendum 23454A, date 02.02.1995 GLP, unpublished TOX2003-2031	Y	BAY
AIIA-5.8.2	Sheets, L.P.	2001	A developmental neurotoxicity screening study with technical grade imidacloprid in Wistar rats. 110245 ! 99-072-DV GLP, unpublished TOX2003-2034	Y	BAY
AIIA-5.8.2	Sheets, L.P. and Hamilton, B.F.	1994	A subchronic dietary neurotoxicity screening study with technical grade imidacloprid (NTN 33893) in Fischer 344 rats. BC7331 GLP, unpublished TOX2003-2033	Y	BAY
AIIA-5.8.2	Sheets, L.P. and Hamilton, B.F.	1994	An acute oral neurotoxicity screening study with technical grade imidacloprid (NTN 33893) in rats. BC7221 GLP, unpublished TOX2003-2032	Y	BAY
AIIA-5.9.1	Faul, J. and Krauthausen, E.	1996	NTN 33893 - Occupational medical experience. MO-00-005246 not GLP, unpublished TOX2003-2036	Y	BAY
AIIA-5.9.1	Faul, J. and Neukaeter	1996	NTN 33893 - Occupational medical experience. MO-00-005257 not GLP, unpublished TOX2003-2037	Y	BAY
AIIA-5.9.1	Steffens, W.	2000	Final report on the poisoning incident "Lizetan Kombistäbchen, 30.5.00". MO-00-014602 not GLP, unpublished TOX2003-2038	Y	BAY

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AIIA-5.9.1	Watanabe, M.	1995	Effects of activated charcoal treatment on acute poisoning by imidacloprid. 23953 ! RP 94004 not GLP, unpublished TOX2003-2017	Y	BAY
AIIIA-7.1.1	Bomann, W.	1995	NTN 33893 600 FS 03905/0711 - Study for acute oral toxicity in rats. REPORT NO.: 24128 GLP, unpublished TOX96-50611	Y	BAY
AIIIA-7.1.1	Bomann, W.	1990	NTN 33893 200 SL 03833/0047 (imidacloprid (proposed)) - Study for acute oral toxicity in rats. 19499 ! T 5034819 GLP, unpublished TOX2003-2081	Y	BAY
AIIIA-7.1.2	Bomann, W.	1995	NTN 33893 600 FS 03905/0711 - Study for acute dermal toxicity in rats. REPORT NO.: 23946 GLP, unpublished TOX96-50612	Y	BAY
AIIIA-7.1.2	Bomann, W.	1990	NTN 33893 200 SL 03833/0047 c.n.: Imidac-loprid (proposed) - Study for acute demal toxicity in rats. 19532 ! T 7034820 GLP, unpublished TOX2003-2082	Y	BAY
AIIIA-7.1.3	Maertins, T.	1990	NTN 33893 200 SL 03833/0047 (c.n. imidac-loprid (proposed)) - Acute inhalation toxicity in the rat. 19598 ! T 8034821 GLP, unpublished TOX2003-2083	Y	BAY
AIIIA-7.1.3	Pauluhn, J.	1996	NTN 33893 600 FS 03905/0711 - Study on acute inhalation toxicity in rats according to OECD no. 403. REPORT NO.: 24578 GLP, unpublished TOX96-50613	N	BAY
AIIIA-7.1.4; AIIIA-7.1.5	Kroetlinger, F.	1994	NTN 33893 600 FS 03905/0711 - Study for skin and eye irritation/corrosion in rabbits. REPORT NO.: 23289 GLP, unpublished TOX96-50614	Y	BAY

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AIIIA-7.1.4; AIIIA-7.1.5	Maertins, T.	1990	NTN 33893 200 SL 03833/0047 (c.n.: Imidacloprid, proposed) - Study for skin and eye irritation/corrosion in rabbits. 19035 ! T 5034828 GLP, unpublished TOX2003-2084	Y	BAY
AIIIA-7.1.6	Diesing, L. and Dreist, M.	1991	NTN 33893 200 SL 03833/0081 (c.n. imidacloprid (proposed) - Studies on skin sensitising effect in guinea pigs (Buehler test). 20456 ! T 5039959 GLP, unpublished TOX2003-2085	Y	BAY
AIIIA-7.1.6	Vohr, H.W.	2001	NTN 33893 600 FS - Study for the skin sensitisation effect in guinea pigs (guinea pig maximisation test according to Magnusson and Kligman). 30820 ! T 3070033 GLP, unpublished TOX2003-2097	Y	BAY
AIIIA-7.2.1	Lundehn, J.R., Westphal, D. et al.	1992	Uniform principles for safeguarding the health of applicators of plant protection roducts (Uniform principles for operator protection). Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirschaft, Berlin-Dahlem, no. 277, 277, 1992, 112 MO-99-018858 not GLP, published TOX2003-430	N	-
AIIIA-7.2.1.1	Anonymous	1992	UK predictive operator exposure model (POEM): A users guide. PSD - Pesticides Safety Directorate, 1992 MO-01-012223 Not GLP, published TOX2002-444	N	-
AIIIA-7.2.1.1	Anonymous	1986	UK predictive operator exposure model (POEM): Estimation of exposure and absorption of pesticides by spray operators. UK Scientific Subcommittee on Pesticides & British Agrochemical Association Joint Medical Panel, 1986 MO-01-012249 GLP, published TOX2002-443	N	-
AIIA-7.2.1.1; AIIIA-7.2.1.2	Mich, G.	1996	Operator exposure in greenhouses during practical use of plant protection products. EF 94-02-03 GLP, unpublished TOX97-50938	Y	BAY

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AIIIA-7.2.2	Ganzelmeier, H., Rautmann, D., Spangenberg, R., Strelke, M., Herrmann, M., Wenzelburger, H.J. and Walter, H.-F.	1995	Studies on the spray drift of plant protection products. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft; Berlin-Dahlem; Heft 305 (English); Blackwell Wissenschafts-Verlag GmbH Berlin/Wien; Kurfürstendamm 57; D-10707 Berlin, 305, 1995, 1-113 MO-98-000356 not GLP, published TOX2003-438	N	-
AIIIA-7.2.2; AIIIA-10.2; AIIIA-10.5; AIIIA-10.8	Ganzelmeier, H., Rautmann, D., Spangenberg, R., Strelke, M., Herrmann, M., Wenzelburger, H.J. and Walter, H.-F.	1995	Studies on the spray drift of plant protection products. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft; Berlin-Dahlem; Heft 305 (English); Blackwell Wissenschafts-Verlag GmbH Berlin/Wien; Kurfürstendamm 57; D-10707 Berlin, 111 MO-98-000356 not GLP, published ANA2003-417 PFL2003-229 WAT2003-682	N	-
AIIIA-7.2.3	Brouwer, D.H., de Haan, M. and van Hemmen, J.J.	2001	Modeling re-entry exposure estimates: Techniques and application rates. Journ.Worker exposure to agrochem., 119, 2001, 119-138 MO-03-011549 not GLP, published TOX2003-2089	N	-
AIIIA-7.2.3	Formoli, T.A. and Fong, H.R.	1993	Estimation of exposure products that contain azinphos-methyl. HS-1650 not GLP, unpublished TOX2003-2091	Y	BAY
AIIIA-7.2.3	van Hemmen, J.J., Chester, G., Hamey, P., Kangas, J., Kirknel, E., Maasfeld, W., Perkins, J., Phillips, J. and Rosario, C.	2002	Post-application exposure of workers to pesticides in agriculture. FAIR3-CT96-1406 not GLP, unpublished TOX2003-2092	Y	BAY

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AIIIA-7.2.3	Krebs, B., Maasfeld, W., Schrader, J. and Wolf, R.	1996	Uniform principles for safeguarding the health of workers re-entring crop growning areas after application of plant protection products. MO-98-000362 ! Ks 950822 GLP, unpublished TOX2003-2088	Y	BAY
AIIIA-7.2.3	Krieger, R.I., Ross, J.H. and Thongsinthusak, T.	1992	Assessing human exposure to pesticides. MO-02-004605 not GLP, published TOX2003-2090	N	-

Codes of owner

BAY: Bayer CropScience Deutschland GmbH