Table A6.8.1/02-1: Rabbit developmental toxicity _Maternal effects

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 A6.8.1.1/02-2: Rat developmental toxicity _Litter effects

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 ↓

Appendix 1: CA-Tables

CA-Table 1: Body weight of dams – absolute and relative to control

	Dose level (mg/kg bw/d)			
	0	**8**	**24**	**72**
Day 0	3348 g	3286 g (98 %)	3222 g (96 %)	3239 g (97 %)
Day 6	3502 g	3477 g (99 %)	3383 g (97 %)	3427 g (98 %)
Day 18	3638 g	3577 g (98 %)	3436 g (94 %)	**3258 g (90 %)****
Day 20	3668 g	3635 g (99 %)	3523 g (96 %)	**3253 g (89 %)****
Day 28	3740 g	3690 g (99 %)	3597 g (96 %)	3523 g (94 %)

** $p \leq 0.01$, Dunnett-test based on pooled variance

CA-Table 2: Rabbit developmental toxicity – Abnormalities (litters/foetuses)

	Dose level (mg/kg bw/d)			
Finding	**0**	**8**	**24**	**72**
Partially fused ribs	1/1	-/-	-/-	-/-
Missing sternebrae	-/-	1/1	-/-	1/2
Hydrocephalus internus	-/-	-/-	1/1	-/-
Shortened tail, no ossified bone in remaining stump	-/-	-/-	-/-	1/1

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 *Multigeneration reproduction study in rats*

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.6.1, II A, 5.6.1 /02*	
	Authors (year)	[redacted] (1990)	
	Title	Multiple generation reproduction study with NTN 33893 technical in rats	
	Company, report No.	Bayer CropScience AG, Report-No.: R5097 BES Ref. : M-027300-03-1	
	Date	1990-06-21, Amended 1992-03-03	
	Testing facility	[redacted]	
	Dates of work	November 1987 – February 1989	
	Test substance(s)	Molecule(s): imidacloprid Substance(s): NTN 33893 Z (Batch-No.: 180587)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 416; FIFRA §83-4; Guidance on Toxicology Data No. 4200, Japan.	
2.2	**GLP**	Yes (certified laboratory	
2.3	**Deviations**	No QAU inspections of analytical work and triglyceride determinations	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Imidacloprid technical, mixed batch no. 180587, purity 94.4 % - 95.3 %	
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.	
3.1.2.1	Description		
3.1.2.2	Purity		
3.1.2.3	Stability		
3.2	**Test Animals**		
3.2.1	Species	Male and female Wistar/HAN rats (Strain Kfm:WIST; Breeder [redacted])	
3.2.2	Strain		
3.2.3	Source		
3.2.4	Sex		
3.2.5	Age/weight at study initiation	123-169 g males / 81-137 g females / 4 weeks old (parent generation)	
3.2.6	Number of animals per group	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.	
3.2.7	Mating	Per OECD 416; FIFRA §83-4; Guidance on Toxicology Data No. 4200,	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 *Multigeneration reproduction study in rats*

3.2.8	Duration of mating	Japan, no deviations noted by the RMS in the December 2005 91/414 DAR	
3.2.9	Deviations from standard protocol	None noted	
3.2.10	Control animals	Yes	
3.3	**Administration/ Exposure**		
3.3.1	Animal assignment to dosage groups	Imidacloprid was administered in the diet at concentrations of 0, 100, 250 and 700 ppm during an 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. See Table A6.8.2/01-1 for test substance intake.	
3.3.2	Duration of exposure before mating		
3.3.3	Duration of exposure in general P, F1, F2 males, females		
Oral			
3.3.4	Type		
3.3.5	Concentration		
3.3.6	Controls	Plain diet	
3.4	**Examinations**	Per OECD 416; FIFRA §83-4; Guidance on Toxicology Data No. 4200, Japan, no deviations noted by the RMS in the December 2005 91/414 DAR	
3.4.1	Clinical signs		
3.4.2	Body weight		
3.4.3	Food/water consumption		
3.4.4	Oestrus cycle		
3.4.5	Sperm parameters		
3.4.6	Offspring		
3.4.7	Organ weights P and F1		
3.4.8	Histopathology P and F1		
3.4.9	Histopathology F1 not selected for mating, F2		
3.5	**Further remarks**	See results for details of examinations	
		4 RESULTS AND DISCUSSION	
4.1	**Effects**		
4.1.1	Parent males	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. See Table A6.8.2/01-2 – Table A6.8.2/01-3 for parental findings.	X
4.1.2	Parent females		X
4.1.3	F1 males		X
4.1.4	F1 females		X
4.1.5	F2 males	There were no treatment-related effects on haematological parameters	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 *Multigeneration reproduction study in rats*

4.1.6	F2 females	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. See Table A6.8.2/01-4. No gross pathological, organogravimetric or histopathological alterations were apparent in the examined parents at doses up to and including 700 ppm. No treatment-related effects were observed on reproduction parameters. 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.
4.2	**Offspring effects**	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. See Table A6.8.2/01-5 and Table A6.8.2/01-6 for offspring findings. 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. 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.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to OECD 416; FIFRA §83-4; Guidance on Toxicology Data No. 4200, Japan guidelines, 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.

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 *Multigeneration reproduction study in rats*

5.2	**Results and discussion**	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.	X
		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.	X
		No gross pathological, organogravimetric or histopathological alterations were apparent in the examined parents at doses up to and including 700 ppm.	
		No treatment-related effects were observed on reproduction parameters. 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.	
		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.	
		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.	
5.3	**Conclusion**		
5.3.1	LO(A)EL parental	700 ppm based on reduced body weight gain	
5.3.2	NO(A)EL parental	250 ppm, equivalent to approximately 20 mg/kg bw/day during the premating period	
5.3.3	LO(A)EL reproduction	>700 ppm	
5.3.4	NO(A)EL reproduction	700 ppm, equivalent to approximately 50 mg/kg bw/day during premating period and pregnancy	
5.3.5	LO(A)EL development	700 ppm based on reduced body weight gains in pups	
5.3.6	NO(A)EL development	250 ppm, equivalent to approximately 40 mg/kg bw/day during lactation	
5.3.7	Reliability	1	
5.3.8	Deficiencies	No	

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA6.8.2 *Multigeneration reproduction study in rats*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/15
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.1.1/4.1.2/5.2 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 intake of F1 generation females was decreased. Since these changes were marginal (< 10%), they were not considered adverse. See Table A6.8.2/01-2 – Table A6.8.2/01-3 for parental findings. 4.1.3/4.1.4/5.2 Elevated cytochrome P-450 and N-demethylase values were determined in males at the 700 ppm dose level, and increased O-demethylase activities in males and females. Elevated O-demethylase values were also found in the F_1 females at 250 ppm. As there was no clear dose dependency in these enzyme level changes and as no other clinical chemistry or histopathological/organ weight liver changes indicative for organ toxicity were observed, these findings are not considered adverse.
Conclusion	The applicant's version is acceptable except for the NOAEL(developmental). In summary, the following NOAEL were obtained: NOAEL(parental): 20 mg/kg bw/d (250 ppm), based on decreased food consumption and body weight gain of dams at 50 mg/kg bw/d (700 ppm) NOAEL(reproduction): 50 mg/kg bw/d (700 ppm), based on the absence of effects at the highest dose level tested NOAEL(offspring): 20 mg/kg bw/d (250 ppm), based on decreased food consumption and body weight gain of pups at 50 mg/kg bw/d
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.8.2/01-1: 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

Table A6.8.2/01-2: 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 A6.8.2/01-3: Two-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

Table A6.8.2/01-3: Two-generation study in rats – Parental findings (F1-generation), continued

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

Table A6.8.2/01-4: 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)

Table A6.8.2/01-5: 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 cn pooled variance)

Table A6.8.2/01-6: 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)

Section A6.9/01 Neurotoxicity Study

Annex Point IIA6.9

Acute oral neurotoxicity study in the rat

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1 REFERENCE

1.1	**Reference**	*PPP monograph B.7.1.1, II A, 5.8.2.1.1 /01*
	Authors (year)	[redacted] (1994a)
	Title	An acute oral neurotoxicity screening study with technical grade imidacloprid (NTN 33893) in rats
	Company, report No.	Bayer CropScience AG, Report-No.: BC7221 BES Ref. : M-028815-02-1
	Date	1994-02-16, Amended: 1994-06-07
	Testing facility	[redacted]
	Dates of work	April 1994
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 2030030)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Imidacloprid, batch no. 2030030, purity: 97.6 % - 98.8 %
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Test Animals**	
3.2.1	Species	Sprague Dawley rats (Strain Sas:CD(SD)BR; Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	Male and female
3.2.5	Number of animals per group	18/sex/dose level
3.2.6	Control animals	Yes

Section A6.9/01 Neurotoxicity Study

Annex Point IIA6.9

Acute oral neurotoxicity study in the rat

3.3	**Administration**	
3.3.1	Exposure	Imidacloprid was administered by gavage in a single dose to fasted Sprague Dawley rats 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.
3.3.2	Dose Levels	
3.3.3	Vehicle	
3.3.4	Concentration in vehicle	
3.3.5	Total volume applied	
3.3.6	Postexposure period	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.
3.3.7	Controls	
3.4	**Examinations**	
3.4.1	Body Weight	Yes
3.4.2	Signs of Toxicity	clinical observations, mortality
3.4.3	Observation schedule	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.
3.4.4	Clinical Chemistry	Yes
3.4.5	Pathology	Yes determination of brain weight, and a gross necropsy.
3.4.6	Histopathology	Yes, Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from central nervous system were examined histopathologically.
3.4.7	Other	The following measurements were performed in the study: automated measurements of activity (figure-eight maze), a functional observational battery
3.4.8	Statistics	ANOVA, Dunnett's test when appropriate

4 RESULTS AND DISCUSSION

4.1	**Body Weight**	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 highdose 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.
4.2	**Clinical signs of toxicity**	

Section A6.9/01 Neurotoxicity Study

Annex Point IIA6.9

Acute oral neurotoxicity study in the rat

4.3	**Clinical Chemistry**	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.	
4.4	**Pathology**	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.	
4.5	**Histopathology**		
4.6	**Other**	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. A dose-related decrease in a measure of motor and locomotor activity was observed in both sexes, with reduced activity in males at the two higher doses and in females at all three doses on day 0. The slightly reduced motor activity in females at 42 mg/kg bw (73% of control) was comparable to that seen before treatment (79% of control) and also 14 days after treatment (69% of control). In addition, 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 (see Table A6.9/01-1). Therefore the slight decreases in the mean values of the activity parameters observed at the dose of 42 mg/kg bw, 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.	X

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a single dose neurotoxicity study in rat conducted according to EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617 guideline, 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. 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.	

Section A6.9/01 Neurotoxicity Study

Annex Point IIA6.9

Acute oral neurotoxicity study in the rat

5.2	**Results and discussion**	Four high-dose males and ten high-dose females died due to treatment, either on the day of treatment or within the day following treatment. 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. In functional observational battery (FOB) and motor and locomotor activity examinations, animals treated with 307 mg/kg bw showed diminished grip strength on treatment day 0. A dose-related decrease in a measure of motor and locomotor activity was observed in both sexes, with reduced activity in males at the two higher doses and in females at all three doses on day 0. The slightly reduced motor activity in females at 42 mg/kg bw (73% of control) was comparable to that seen before treatment (79% of control) and also 14 days after treatment (69% of control). In addition, 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. Therefore the slight decreases in the mean values of the activity parameters observed at the dose of 42 mg/kg bw, 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. 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. 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.	X
5.3	**Conclusion**	No evidence of a specific neurotoxic potential was seen.	
5.3.1	LOAEL	151 mg/kg bw based on clinical signs and behavioural effects	
5.3.2	NOAEL	42 mg/kg bw	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Section A6.9/01 Neurotoxicity Study

Annex Point IIA6.9 *Acute oral neurotoxicity study in the rat*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/07
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable. 4.6/5.2 See CA-Tables 1 and 2 for details on motor and locomotor activities in females.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.9/01-1: 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

Appendix 1: CA-Tables

CA-Table 1: Motor activities of female rats (pretreatment, day 0, day 7, and day 14)

Group	Pretreatment	Day 0	Day 7	Day 14
0 MG/KG	1054 ±390 (12)	504 ±262 (12)	840 ±388 (12)	970 ±326 (12)
50 MG/KG	831 ±275 (12)	366 ±194 (12)	769 ±251 (12)	665 ±254 (12)
150 MG/KG	880 ±293 (12)	263* ± 93 (12)	725 ±303 (12)	784 ±283 (12)
350 MG/KG	990 ±248 (12)	96* ± 71 (10)	897 ±130 (4)	853 ±137 (4)

*Significantly different from control (p≤0.05, ANOVA)
Mean ± S.D (n) for 1:30:00 (hh:mm:ss) Test Session
Nominal Day 0 = 30NOV92

CA-Table 2: Locomotor activities of female rats (pretreatment, day 0, day 7, and day 14)

Group	Pretreatment	Day 0	Day 7	Day 14
0 MG/KG	433 ±194 (12)	166 ± 84 (12)	343 ±187 (12)	370 ±160 (12)
50 MG/KG	375 ±138 (12)	124 ± 46 (12)	326 ±141 (12)	293 ±140 (12)
150 MG/KG	349 ±141 (12)	89 ± 11 (12)	304 ±140 (12)	327 ±153 (12)
350 MG/KG	368 ± 95 (12)	18 ± 30 (10)	334 ± 53 (4)	332 ± 80 (4)

*Significantly different from control (p≤0.05, ANOVA)
Mean ± S.D (n) for 1:30:00 (hh:mm:ss) Test Session
Nominal Day 0 = 30NOV92

Section A6.9/02 Neurotoxicity Study

Annex Point IIA6.9

Subchronic neurotoxicity study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph B.7.1.1, II A, 5.8.2.1.2 /01*
	Authors (year)	[redacted] (1994b)
	Title	A subchronic dietary neurotoxicity screening study with technical grade Imidacloprid (NTN 33893) in Fischer 344 rats
	Company, report No.	Bayer CropScience AG, Report-No.: BC7331 BES Ref. : M-027944-01-1
	Date	1994-06-13
	Testing facility	[redacted]
	Dates of work	January - April 1993
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 2030030)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Imidacloprid, batch no. 2030030, purity: 97.6 % - 98.8 %
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.

Section A6.9/02 **Neurotoxicity Study**

Annex Point IIA6.9 *Subchronic neurotoxicity study in the rat*

3.1.2.1	Purity		
3.1.2.2	Description		
3.1.2.3	Stability		
3.2	**Test Animals**		
3.2.1	Species	Fischer 344 rats (Strain CDF(F-344)/BR; Breeder [redacted])	
3.2.2	Strain		
3.2.3	Source		
3.2.4	Sex	Male and female	
3.2.5	Number of animals per group	18/sex/dose level	
3.2.6	Control animals	Yes	
3.3	**Administration**		
3.3.1	Exposure	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.	
3.3.2	Dose Levels		
3.3.3	Postexposure period		
3.3.4	Controls		
3.4	**Examinations**		
3.4.1	Body Weight	Yes	
3.4.2	Signs of Toxicity	clinical observations, mortality, food consumption	
3.4.3	Observation schedule	automated measurements of activity (figure-eight maze), functional observation battery according to guideline requirements; no deviations noted by the RMS in the December 2005 91/414 draft DAR	
3.4.4	Clinical Chemistry	Yes	
3.4.5	Pathology	Yes determination of brain weight, and a gross necropsy.	
3.4.6	Histopathology	Yes, Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from central nervous system were examined histopathologically.	
		4 RESULTS AND DISCUSSION	
4.1	**Body Weight**	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.	X
4.2	**Clinical signs of toxicity**		
4.3	**Ophthalmoscopy**	No compound-related ophthalmic findings.	
4.4	**Clinical Chemistry**	Decreased triglyceride levels, lactate dehydrogenase and creatine kinase activities were established for the middle and high dose group.	X
4.5	**Pathology**	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.	
4.6	**Histopathology**		

Section A6.9/02 Neurotoxicity Study

Annex Point IIA6.9

Subchronic neurotoxicity study in the rat

4.7	**Other**	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.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a repeat dose neurotoxicity study in rat conducted according to EPA-FIFRA, Addendum 10, EPA 540/09-91-123, PB 91-154617 guideline, 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, 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.	
5.2	**Results and discussion**	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 findings. Decreased triglyceride levels, lactate dehydrogenase and creatine kinase activities were established for the middle and high dose group. 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. 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.	X
5.3	**Conclusion**		
5.3.1	LOAEL	Overall: 963 ppm, equivalent to 63.3 mg/kg bw/day males and 69.3 for females, based on reduced body weights and food consumption at 963 ppm	

Section A6.9/02 Neurotoxicity Study

Annex Point IIA6.9 *Subchronic neurotoxicity study in the rat*

5.3.2	NOAEL	Overall: 140 ppm, equivalent to 9.3 mg/kg bw/day males and 10.5 for females Neurotoxicity: 3027 ppm, equivalent to 196 mg/kg bw/day males and 213 for females (highest dose tested)
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/15
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.1/5.2 Body weight was reduced by more than 10 % compared to controls at a dose level of 196 mg/kg bw/d (males only). 4.4/5.2. Creatine kinase levels in males and females and lactate dehydrogenase in females were decreased at the mid and high dose levels. In the high dose groups, triglyceride and phosphate levels were decreased in both sexes as well as total protein and albumin in females.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Appendix 1: CA-Tables

CA-Table 1: Body weight – relative to control

Week		0	1	2	3	4	5	6	7	8	9	10	11	12	13
Males															
Dose level (mg/kg bw/d)	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	9	100	100	100	101	101	101	101	101	101	101	101	101	101	101
	63	100	98	96	96	94	91	92	92	93	93	93	92	93	92
	196	99	92	89	88	85	83	84	83	84	85	85	85	84	84
Females															
Dose level (mg/kg bw/d)	0	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	11	100	99	98	98	98	98	98	98	98	98	98	98	98	99
	69	99	98	96	96	96	95	95	95	96	96	97	97	97	97
	213	98	96	95	95	94	93	92	92	92	92	92	92	92	92

CA-Table 2: Righting reflex – male rats, wk 13

Dose level (ppm)		0	150	1000	3000
Number of Animals Examined		12	12	12	12
Righting Reflex	Normal Landing	11	10	9	5*
	Slightly Uncoordinated	1	2	3	7*

* Statistically different from control ($P < 0.05$)

CA-Table 3: Body weight, grip strength (kg) and footsplay (mm) for male rats, wk 8 (mean ± SD)

Dose level (ppm)	Body weight	Grip strength		
		Forelimb	Hindlimb	Footsplay
0	261 ± 10	0.94 ± 0.14	0.34 ± 0.07	65 ± 6
150	264 ± 14	0.87 ± 0.16	0.34 ± 0.05	64 ± 11
1000	240* ± 17	0.82 ± 0.12	0.34 ± 0.10	63 ± 13
3000	217* ± 10	0.72* ± 0.14	0.29 ± 0.05	56 ± 10

* Statistically different from control ($P < 0.05$)

Section A6.9/03

Annex Point IIA6.9

Neurotoxicity Study

Developmental neurotoxicity study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph B.7.1.1, II A, 5.8.2.1.3 /01*
	Authors (year)	[redacted] (2001)
	Title	A developmental neurotoxicity screening study with technical grade Imidacloprid in Wistar rats
	Company, report No.	Bayer CropScience AG, Report-No.: 110245 BES Ref. : M-084646-01-1
	Date	14.09.2001
	Testing facility	[redacted]
	Dates of work	June – October 1999
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 803-0273)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OPTTS 870.6300
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	The period of compound administration was extended (from gestation day 0 through lactaion day 21, rather than from gestation day 6 to lactation day 10 as requested in the guideline). This change does not alter the validity of the study.

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Imidacloprid, batch no. 803-0273, purity: 98.2 – 98.4 %
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.

Section A6.9/03 Neurotoxicity Study

Annex Point IIA6.9 *Developmental neurotoxicity study in the rat*

3.1.2.1	Purity	
3.1.2.2	Description	
3.1.2.3	Stability	
3.2	**Test Animals**	
3.2.1	Species	female Wistar rats (Strain Crl:W(HAN)BR, Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Number of animals per group	30/dose level
3.2.6	Control animals	Yes
3.3	**Administration**	
3.3.1	Exposure	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 A.6.9/03-1.
3.3.2	Dose Levels	
3.3.3	Postexposure period	
3.3.4	Controls	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.
3.4	**Examinations**	Subsets of surviving offspring, representing at least 20 litters per level, were subjected to evaluation
3.4.1	Body Weight	Yes
3.4.2	Signs of Toxicity	clinical observations including an abbreviated FOB, body weight, food consumption, developmental landmarks
3.4.3	Observation schedule	automated measurements of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance and a water maze task) and ophthalmic examination. according to guideline requirements; no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.4.4	Pathology	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).
3.4.5	Histopathology	

Section A6.9/03 **Neurotoxicity Study**

Annex Point IIA6.9 *Developmental neurotoxicity study in the rat*

		4 RESULTS AND DISCUSSION	
4.1	**Body Weight**	See Table A6.9/03-2 for a summary of maternal data. 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.	X
4.2	**Clinical signs of toxicity**	See Table A6.9/03-3 for a summary of offspring data. 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.	
4.3	**Ophthalmoscopy**	No compound-related lesions evident at any dietary level for offspring.	
4.4	**Pathology**	No morphologic changes in neural tissues were seen in the histopathological investigations. Brain weight was not affected at any dose level.	
4.5	**Histopathology**		
4.6	**Other**	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.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a developmental neurotoxicity study in rat conducted according to EPA-FIFRA OPPTS 870:6300 guideline, 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. 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).	

Section A6.9/03 Neurotoxicity Study

Annex Point IIA6.9 *Developmental neurotoxicity study in the rat*

5.2	**Results and discussion**	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. 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. Ophthalmoscopic examination did not reveal compound-related findings. 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.	X
5.3	**Conclusion**	No permanent neurotoxic effects of imidacloprid were detected in developing rats.	
5.3.1	LOAEL	691 ppm based on decreased food consumption in the dams and on retarded body weight gain of pups and decreased motor/locomotor activity	X
5.3.2	NOAEL	227 ppm (maternal and offspring)	X
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/10/04
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.1/5.2 Maternal: There were no compound-related clinical signs or effects on body weight, reproduction parameters, and in the FOB. Food consumption was lower at 691 ppm during the last week of pregnancy and the first week of lactation, which was not considered an adverse effect.
Conclusion	NOAEL(offspring): 227 ppm, equivalent to ca. 30 mg/kg bw/d (the lower boundary of the maternal dose level range during lactation) based on retarded body wt gain (PND 4-21) and decreased motor/ locomotor activity of pups at a maternal dose level of 691 ppm. These effects are however considered direct neurotoxic and not developmental effects. NOAEL(maternal): 691 ppm equivalent to ca. 80-155 mg/kg bw/d, the highest dose level tested.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A.6.9/03-1: 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

Table A.6.9/03-2: 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

Table A.6.9/03-3 : 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)

Section A6.12.1/01 Human Case Report

Annex Point IIA6.12 *Occupational medical surveillance*

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] Occupational Medical Experiences with Imidacloprid, report by [redacted], 5 November 2004. reference MO-05-004265 - BES Ref. : M-245951-01-1

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Imidacloprid active substance
3.2	**Persons exposed**	
3.2.1	Sex	Not given
3.2.2	Age/weight	Not given
3.2.3	Known Diseases	Not given
3.2.4	Number of persons	65
3.2.5	Other information	Personal safety measures are safety glasses, rubber gloves, instructions to avoid skin contact
3.3	**Exposure**	Assume all exposures are relevant in an occupational situation
3.3.1	Reason of exposure	Occupational
3.3.2	Frequency of exposure	Multiple
3.3.3	Overall time period of exposure	Production has been in place since 1993
3.3.4	Duration of single exposure	Not relevant
3.3.5	Exposure concentration/dose	not available
3.3.6	Other information	More than 15,000 mt of a.s. produced since 1993
3.4	**Examinations**	Occupational medical surveillance of workers performed every 2 years. Medical exams: History, full physical examination with orienting neurological status (reflexes, sensitivity, coordination), skin status Lab exams: BSR, full blood count, AST, ALT, y-GT, glucose, creatinine, cholesterol, urine status Technical exams: lung function, ecg/ergometry, vision, audiometry, chest x-ray, sonography (if necessary)
3.5	**Treatment**	Not applicable

Section A6.12.1/01 Human Case Report

Annex Point IIA6.12 *Occupational medical surveillance*

4 RESULTS

4.1	**Clinical Signs**	None to date of report
4.2	**Results of examinations**	No undesirable findings in any of the examinations
4.3	**Effectivity of medical treatment**	Not applicable
4.4	**Outcome**	No unwanted effects directly linked to imidacloprid occupational exposure
4.5	**Other**	No imidacloprid-related allergenicity observations have been determined since 1993

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	At least 65 workers have been occupationally exposed to imidacloprid active substance in the production facility. Occupational medical surveillance of workers has been performed every 2 years. Medical exams: History, full physical examination with orienting neurological status (reflexes, sensitivity, coordination), skin status Lab exams: BSR, full blood count, AST, ALT, y-GT, glucose, creatinine, cholesterol, urine status Technical exams: lung function, ecg/ergometry, vision, audiometry, chest x-ray, sonography (if necessary)
5.2	**Results and discussion**	No undesirable findings have been reported in any of the examinations
5.3	**Conclusion**	Since 1993 no accidents have occurred in workers in the active substance production facility and no consultations have been required due to work or contact with imidacloprid. No imidacloporid related allergenicity observations have been made.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.12.1/02 Human Case Report

Annex Point IIA6.12 *Occupational medical surveillance*

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] Occupational Medical Experiences with Imidacloprid, Gel 2.15% report by [redacted] on behalf of Bayer AG BES Ref.: M-267506-01-1 6.12/02

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	Imidacloprid
3.2	**Persons exposed**	Plant operatives during formulation
3.2.1	Sex	Not given
3.2.2	Age/weight	Not given
3.2.3	Known Diseases	Not given
3.2.4	Number of persons	14
3.2.5	Other information	Personal safety measures are: Generally: cap, safety goggles, certified permeable pharmaceutical clothes, safety shoes Handling ingredients and open product: mask (3M, 9332), gloves (UVEX Rubifix S or UVEX S6 Profabutyl Filling/packaging: safety goggles, certified permeable pharmaceutical clothes, safety shoes
3.3	**Exposure**	Assume all exposures are relevant in an occupational situation
3.3.1	Reason of exposure	Occupational: potential exposure during formulation
3.3.2	Frequency of exposure	Multiple
3.3.3	Overall time period of exposure	Years 1998 - 2005
3.3.4	Duration of single exposure	Not specified
3.3.5	Exposure concentration/dose	Not specified
3.3.6	Other information	122 200 Kg. produced from 1998 - 2005

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Annex Point IIA6.12 *Occupational medical surveillance*

3.4	**Examinations**	Occupational medical surveillance of workers performed at commencement of employment and every 3 years. Medical exams: History, full physical examination with orienting neurological status (reflexes, sensitivity, coordination), skin status Examination based on the German rules G25 (driving/steering), G26.2 (breathing protection), G37 (VDU work), B04 (BAPRO). Lab exams: BSR, full blood count, AST, ALT, y-GT, glucose, creatinine, cholesterol, urine status Technical exams: lung function, vision testing, audiometry	X
3.5	**Treatment**	Not applicable	
		4 RESULTS	
4.1	**Clinical Signs**	None to date of report	
4.2	**Results of examinations**	No undesirable findings in any of the examinations	
4.3	**Effectivity of medical treatment**	Not applicable	
4.4	**Outcome**	No unwanted effects directly linked to imidacloprid occupational exposure	
4.5	**Other**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	At least 14 workers have been occupationally exposed to imidacloprid active substance during formulation of Imidacloprid GL 2.15. Occupational medical surveillance of workers has been performed every 3 years. Medical exams: History, full physical examination with orienting neurological status (reflexes, sensitivity, coordination), skin status, and Examination based on the German rules G25 (driving/steering), G26.2 (breathing protection), G37 (VDU work), B04 (BAPRO). Lab exams: BSR, full blood count, AST, ALT, y-GT, glucose, creatinine, cholesterol, urine status Technical exams: lung function, vision testing, audiometry.	
5.2	**Results and discussion**	No undesirable findings have been reported in any of the examinations	
5.3	**Conclusion**	*During the production period (1998 – 2005)* no accidents with imidacloprid occurred in workers and no consultations were required due to work or contact with Imidacloprid GL 2.15.	

Section A6.12.1/02 Human Case Report

Annex Point IIA6.12 *Occupational medical surveillance*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12 - non professionals
Materials and Methods	3.4 Laboratory analyses were only performed upon commencement of employment, therefore no imidacloprid-related results are available. Otherwise, applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/01

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /05*
	Authors (year)	[redacted] (1991d)
	Title	WAK 3839 - Acute oral toxicity study on rats
	Company, report No.	Bayer CropScience AG, Report-No.: RA91017 BES Ref. : M-028685-01-1
	Date	1991-03-11
	Testing facility	[redacted]
	Dates of work	December 1990 – March 1991
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid WAK 3839 Batch No. TX020390
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.1 % suspended in polyethylene glycol
3.1.2	Specification	stability guaranteed for the duration of the study.
3.1.2.1	Description	
3.1.2.2	Purity	
3.1.2.3	Stability	
3.2	**Test Animals**	Sprague Dawley rats (Strain Crj:CD (SPF); Breeder [redacted]
3.2.1	Species	
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	

Section A6.14/01

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

3.2.5	Age/weight at study initiation	191-227 g for males / 144-161 g for females / 6 weeks old
3.2.6	Number of animals per group	5 male, 5 female
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
3.3.2	Type	NTN 33893-nitrosimine was suspended in polyethylene glycol 400. The dosing solutions were administered by stomach tube to fasted rats at concentrations of 4000, 2500, 1560 and 980 mg/kg bw. Application volume: 10 mL/kg bw
3.3.3	Concentration	
3.3.4	Vehicle	
3.3.5	Concentration in vehicle	
3.3.6	Total volume applied	
3.4	**Examinations**	Clinical signs, gross necropsy
3.5	**Method of determination of LD_{50}**	Method of Thompson
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	See Table A6.14/01-1. Mydriasis, tremor, sedation, exophtalmos, abnormal respiration, emaciation, chromodacryorrhea, nasal bleeding, convulsion, abnormal gait, mortality.
4.2	**Pathology**	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.
4.3	**LD_{50}**	Females 3560 mg/kg bw Males 1980 mg/kg bw

Section A6.14/01

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted according to Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1. guidelines, NTN 33893 nitrosimine was administered in a single dose by oral gavage to fasted Sprague Dawley rats at dose levels ranging from 980-4000 mg/kg bw.
5.2	**Results and discussion**	Mortalities occurred at doses at and above 1560 mg/kg bw in males and at and above 2500 mg/kg bw in females. Clinical signs included mydriasis, tremor, sedation, exophtalmos, abnormal respiration, emaciation, chromodacryorrhea, nasal bleeding, convulsion, abnormal gait. Findings in animals that died during the post-treatment observation period included: 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 test article-related gross pathological findings were observed in the animals sacrificed at the end of the post-treatment observation period.
5.3	**Conclusion**	LD 50 females 3360 mg/kg bw Males 1980 mg/kg bw NTN 33893-nitrosimine is of moderate toxicity to rats following acute oral administration.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	

Section A6.14/01

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.14/01-1. NTN 33893-nitrosimine-Acute Oral Toxicity to Rat

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

Section A6.14/02

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in mouse

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /07*	
	Authors (year)	[redacted] 1988b)	
	Title	NTN 37571 - Acute toxicity study on mice	
	Company, report No.	Bayer CropScience AG, Report-No.: RS88038 BES Ref. : M-028572-01-1	
	Date	1988-10-19	
	Testing facility	[redacted]	
	Dates of work	Not detailed	
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	In main accordance with OECD 401	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	NTN 37571 (NTN 33893-nitrosimine), mixed batch no. TX160888, TX250888, TX060988, purity not reported, dissolved in DMSO and suspended in polyethylene glycol 400.	
3.1.2	Specification		
3.1.2.1	Description		
3.1.2.2	Purity		
3.1.2.3	Stability		

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in mouse

3.2	**Test Animals**	ICR Mice (Strain Crj:CD-1; Breeder [redacted]), male and female
3.2.1	Species	
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	21-29 g for males / 19-23 g for females / 5 weeks old
3.2.6	Number of animals per group	5 male, 5 female
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Postexposure period	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.
3.3.2	Type	
3.3.3	Concentration	
3.3.4	Vehicle	
3.3.5	Concentration in vehicle	
3.3.6	Total volume applied	
3.4	**Examinations**	Clinical signs, gross necropsy
3.5	**Method of determination of LD_{50}**	Not reported

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	See Table A6.14/02-1. Abnormal gait, abnormal respiration, exophthalmos, tremor, convulsion, chick-like vocalization, mortality.
4.2	**Pathology**	No specific findings.
4.3	**LD_{50}**	Males 200 mg/kg bw Females ca. 200 mg/kg bw

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in mouse

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted in main accordance to OECD guidelines, NTN 33893-nitrosimine was administered in a single dose by gavage to ICR mice at dose levels ranging from 100-450 mg/kg bw .	
5.2	**Results and discussion**	Mortalities occurred at doses at and above 200 mg/kg bw in males and at all doses in females. Clinical signs included abnormal gait, abnormal respiration, exophthalmos, tremor, convulsion, chick-like vocalization. There were no specific findings upon necropsy. 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.	
5.3	**Conclusion**	LD50 Males 200 mg/kg bw LD50 Females ca. 200 mg/kg bw Following acute oral administration imidacloprid is more toxic in mice than in rats.	X
5.3.1	Reliability	3	
5.3.2	Deficiencies	Study report is insufficient to evaluate deviations, the study is considered supplementary	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	1.1 WAK 3839, imidacloprid-nitrosimine
Results and discussion	Applicant's version is acceptable.
Conclusion	5.3 LD_{50} ca. 200-300 mg/kg bw Following acute oral administration imidacloprid-nitrosimine is more toxic in mice than in rats.
Reliability	2
Acceptability	Acceptable with restrictions: reporting is partly deficient but overall, results can be used for risk assessment.
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.14/02-1. 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

Section A6.14/03

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /08*	
	Authors (year)	[redacted] (1990b)	
	Title	WAK 3839 - Reverse mutation assay (Salmonella tyhimurium and Escherichia coli)	
	Company, report No.	Bayer CropScience AG, Report-No.: RA90035 BES Ref. : M-028631-01-1	
	Date	1990-11-26	
	Testing facility	[redacted]	
	Dates of work	May – October 1990	
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosamine Batch no. TX020390	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.3 %	
3.1.2	Specification	stability guaranteed for the duration of the study.	
3.1.2.1	Purity		
3.1.2.2	Stability		
3.2	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98 E.coli: WP2 uvr A	
3.2.2	Metabolic activation system	S9 mix	
3.2.3	Positive control	AF2, 2-Aminoanthracene, N-Ethyl-N'-nitro-N-nitrosoguanidine and 9-Aminoacridine	

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid nitrosimine 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. AF2, 2AA, ENNG and 9-AA were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	WAK 3839 concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, NTN 33893 nitrosamine (WAK 3839) 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.
5.2	**Results and discussion**	WAK 3839 concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be non-mutagenic in the salmonella/microsome test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.14/03

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	2.3 Study in accordance without deficiencies to OECD471 (26th May 1983) Deficiencies to OECD471 (21st July 1997): activation activity of S9 solely tested with 2-aminoanthracene
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/04

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the CHO-HGPRT assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /09*
3.	Authors (year)	[redacted] (1989b)
	Title	WAK 3839 - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro
	Company, report No.	Bayer CropScience AG, Report-No.: 17757 BES Ref. : M-027645-01-1
	Date	1989-02-22
4.	Testing facility	[redacted]
5.	Dates of work	August 1988
6.	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosimne Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 476, FIFRA PB 84-233295, 88/302/EEC
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 94.3 %, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	mammalian cell lines: Chinese hamster Ovary (CHO)
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	Ethanemethanesulfonate (EMS) was used as a positive control without S9 mix, and dimethylbenzanthracene (DMBA) as a positive control with S9 mix.

Section A6.14/04

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the CHO-HGPRT assay

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per OECD 476, 88/302/EEC, FIFRA PB 84-233295, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	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.
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an *in vitro* gene mutation assay conducted according to OECD 476, 88/302/EEC, FIFRA PB 84-233295 guidelines, NTN 33893-nitrosimine was evaluated for mutagenic effects at the hypoxanthine-guanine phosphoribosyl transferase locus (forward mutation assay) in Chinese hamster ovary cells after 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.
5.2	**Results and discussion**	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..
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be non-mutagenic in the CHO-HPRT Forward Mutation Assay, both with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.14/04

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the CHO-HGPRT assay

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/05

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the V79-HGPRT assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1/10*
	Authors (year)	[redacted] (1989c)
	Title	WAK 3839 - Mutagenicity study for the detection of induced forward mutations in the V79-HGPRT assay in vitro
	Company, report No.	Bayer CropScience AG, Report-No.: 18281 BES Ref. : M-025757-01-1
	Date	1989-08-15
	Testing facility	[redacted]
	Dates of work	January – April 1989
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosimine Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 476, FIFRA PB 84-233295, 88/302/EEC
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9%, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test
3.2.1	Organism/cell type	mammalian cell lines: Chinese hamster lung cell line V79
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	Ethanemethanesulfonate (EMS) was used as a positive control without S9 mix, and dimethylbenzanthracene (DMBA) as a positive control with S9 mix.

Section A6.14/05

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the V79-HGPRT assay

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per OECD 476, 88/302/EEC, FIFRA PB 84-233295, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without metabolic activation	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.
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an *in vitro* gene mutation assay conducted according to OECD 476, 88/302/EEC, FIFRA PB 84-233295 guidelines, 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.
5.2	**Results and discussion**	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.
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be non-mutagenic in the V79-HPRT Forward Mutation Assay, both with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.14/05

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Forward mutation in the V79-HGPRT assay

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/06

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Rec-assay with spores in the bacterial system

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /11*
	Authors (year)	[redacted] (1991b)
	Title	WAK 3839 - Rec-assay with spores in the bacterial system
	Company, report No.	Bayer CropScience AG, Report-No.: RA91015 BES Ref. : M-028680-01-1
	Date	1991-03-01
	Testing facility	[redacted]
	Dates of work	January 1991
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosimine Batch no. TX020390
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese MAFF Guideline No. 4200
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. TX020390, purity: 98.1%, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	Rec-assay
3.2.1	Organism/cell type	*Bacillus subtilis* strains H17 (rec+) and M 45 (rec-)
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	MMC and 2-AA

Section A6.14/06

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Rec-assay with spores in the bacterial system

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per MAFF Guideline no. 4200, no deviations noted by the RMS in the December 2005 91/414 draft DAR
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	Growth inhibition for both strains were not observed at WAK 3839 concentrations of up to 2000 µg/plate with and without metabolic activation.
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an *in vitro* gene mutation assay conducted according to Japanese MAFF guideline 4200, 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.
5.2	**Results and discussion**	Growth inhibition for both strains were not observed at WAK 3839 concentrations of up to 2000 µg/plate with and without metabolic activation.
5.3	**Conclusion**	NTN 33893-nitrosimine did not induce DNA damage in the Rec-assay.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/07

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Rat primary hepatocyte unscheduled DNA synthesis assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.4.1 /12*
3.	Authors (year)	[redacted] (1989)
	Title	Unscheduled DNA synthesis in primary hepatocytes of male rats *in vitro* with WAK 3839
	Company, report No.	Bayer CropScience AG, Report-No.: R4746 BES Ref. : M-026532-01-1
	Date	1989-04-24
4.	Testing facility	[redacted]
5.	Dates of work	September 1988 – January 1989
6.	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosamine, Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 482; EEC 88/302; EPA FIFRA (1986).
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9 %; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	Unscheduled DNA synthesis in mammalian cells *in vitro*
3.2.1	Organism/cell type	primary culture: hepatocytes
3.2.2	Positive control	2-Acetyl aminofluorene

Section A6.14/07

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Rat primary hepatocyte unscheduled DNA synthesis assay

3.3	**Administration / Exposure; Application of test substance**		
3.3.1	Concentrations	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.	X
3.3.2	Way of application		
3.3.3	Pre-incubation time		
3.3.4	Other modifications		
3.4	**Examinations**	Per OECD 482, EPA FIFRA (1986), 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR	
		4 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**	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.	
4.2	**Cytotoxicity**		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In an *in vitro* gene mutation assay conducted according to OECD 482, FIFRA 1986, 88/302/EEC guidelines, NTN 33893-nitrosimine was tested for mutagenic effects in the 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.	X
5.2	**Results and discussion**	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.	
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be non-mutagenic in the rat primary hepatocyte UDS assay.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/13
Materials and Methods	Applicant's version is acceptable.
Results and discussion	3.3.1/5.1 Rat primary hepatocytes were exposed to NTN 33893-nitrosimine at concentrations from about 0.04 µg/mL to 1333.33 µg/mL.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/08

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

In vitro chromosome aberration assay in Chinese Hamster V79 cells

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /13*
3.	Authors (year)	[redacted] (1989)
	Title	Chromosome aberration assay in Chinese hamster V79 cells *in vitro* with WAK 3839
	Company, report No.	Bayer CropScience AG, Report-No.: R4849 BES Ref. : M-026528-01-1
	Date	1989-09-27
4.	Testing facility	[redacted]
5.	Dates of work	April – July 1989
6.	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosamine Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 473; EEC B.10; EPA FIFRA (1986); Japan (1984).
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.8 %, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	Chromosome aberration assay in mammalian cells
3.2.1	Organism/cell type	mammalian cell lines: Chinese hamster V79 cells
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	Ethylmethanesulfonate (without S9 mix) and cyclophosphamide (with S9 mix)

Section A6.14/08

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

In vitro chromosome aberration assay in Chinese Hamster V79 cells

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986, and Japan (1984) guidelines, no deviations noted by the RMS in the December 2005 91/414 draft DAR
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	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.
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In a study conducted according to OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986, and Japan (1984) guidelines, the *in vitro* potential of NTN 33893-nitrosimine to induce structural chromosome aberrations was tested 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.
5.2	**Results and discussion**	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.
5.3	**Conclusion**	NTN 33893-nitrosimine does not induce structural chromosome aberration in the V79 Chinese hamster cell assay, both with and without metabolic activation.

Section A6.14/08

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

In vitro chromosome aberration assay in Chinese Hamster V79 cells

5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/09

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Mouse micronucleus study after oral application

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /17*
	Authors (year)	[redacted] (1989e)
	Title	WAK 3839 - Micronucleus test on the mouse after oral application
	Company, report No.	Bayer CropScience AG, Report-No.: 18406 BES Ref. : M-025775-01-1
	Date	1989-10-03
	Testing facility	[redacted]
	Dates of work	July 1989
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosamine, Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EEC B.12, OECD 474, US EPS 1984 PB 84-23329
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9%, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.1.2.3	Maximum tolerable dose	100 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Source	NMRI mice (Strain Bor:NMRI (SPF Han); Breeder [redacted]
3.2.3	Sex	Male and female
3.2.4	Age/weight at study initiation	28-40 g / 8-12 weeks old
3.2.5	Control animals	Yes

Section A6.14/09

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Mouse micronucleus study after oral application

3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Postexposure period	24, 48, 72 h after treatment
		Oral
3.3.3	Type	gavage
3.3.4	Vehicle	0.5% aqueous Cremophor emulasion
3.3.5	Concentration in vehicle	Sufficient to deliver the equivalent to 100 mg as/kg bw
3.3.6	Total volume applied	10 ml/kg bw
3.3.7	Substance used as Positive Control	Cyclophophamide (monohydrate), 20 mg/kg bw
3.4	**Examinations**	In accordance with EEC B.12, OECD 474, US EPS 1984 PB 84-23329 guidelines, no deviations noted by the RMS in the December 2005 91/414 DAR
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	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.
4.2	**Genotoxicity**	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.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an *in vivo* clastogenicity study conducted according to EEC B.12, OECD 474, US EPS 1984 PB 84-23329guideline, 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.
5.2	**Results and discussion**	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.
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be negative in the micronucleus test after oral administration.
5.3.1	Reliability	1

Section A6.14/09 **Annex Point IIIAXI.2**	**Toxic effects of substances generated from an active substance** *Mouse micronucleus study after oral application*
5.3.2 Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/10

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Mouse micronucleus study after i.p. application

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /18*
	Authors (year)	[redacted] (1989f)
	Title	WAK 3839 or NTN 37571 - Micronucleus test on the mouse after intraperitoneal injection
	Company, report No.	Bayer CropScience AG, Report-No.: 18407 BES Ref. : M-025706-01-1
	Date	1989-10-03
	Testing facility	[redacted]
	Dates of work	June 1989
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosimine, Batch no. WAK3839/C-E
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EEC B.12, OECD 474, US EPS 1984 PB 84-23329
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), batch no. WAK 3839/C-E, purity: 98.9%, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.1.2.3	Maximum tolerable dose	50 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Source	NMRI mice (Strain Bor:NMRI (SPF Han); Breeder [redacted]
3.2.3	Sex	Male and female
3.2.4	Age/weight at study initiation	31-41 g / 8-12 weeks old

Section A6.14/10

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Mouse micronucleus study after i.p. application

3.2.5	Control animals	Yes
3.3	**Administration/ Exposure**	intraperitoneal
3.3.1	Number of applications	1
3.3.2	Postexposure period	24, 48, 72 h after treatment
		i.p.
3.3.3	Vehicle	0.5% aqueous Cremophor emulasion
3.3.4	Concentration in vehicle	Sufficient to deliver the equivalent to 50 mg as/kg bw
3.3.5	Total volume applied	10 ml/kg bw
3.3.6	Substance used as Positive Control	Cyclophophamide (monohydrate), 20 mg/kg bw
3.4	**Examinations**	In accordance with EEC B.12, OECD 474, US EPS 1984 PB 84-23329 guidelines, no deviations noted by the RMS in the December 2005 91/414 DAR

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	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.
4.2	**Genotoxicity**	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.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an *in vivo* clastogenicity study conducted according to EEC B.12, OECD 474, US EPS 1984 PB 84-23329guideline, NTN 33893-nitrosimine was tested for clastogenic effects using the micronucleus test on the mouse 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. Administration volume was 10 mL/kg bw. Smears were prepared from femoral bone marrow at 24, 48 and 72 hours postdose.
5.2	**Results and discussion**	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.
5.3	**Conclusion**	NTN 33893-nitrosimine is considered to be negative in the micronucleus test after intraperitoneal administration.
5.3.1	Reliability	1

Section A6.14/10 **Annex Point IIIAXI.2**	**Toxic effects of substances generated from an active substance** *Mouse micronucleus study after i.p. application*
5.3.2 Deficiencies	No

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/11

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Subchronic toxicity study in rat (administration in drinking water)

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP Monograph B6.8.1, II A, 5.8.1 /19*	
	Authors (year)	[redacted] (1992)	
	Title	WAK 3839 - Subchronic toxicological study on rats (twelve-week administration on drinking water)	
	Company, report No.	Bayer CropScience AG, Report-No.: 21140 BES Ref. : M-029731-01-1	
	Date	1992-03-02	
	Testing facility	[redacted]	
	Dates of work	September – December 1989	
	Test substance(s)	Molecule(s): WAK 3839, imidacloprid nitrosamine	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 408	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	WAK 3839 (NTN 33893-nitrosimine), various batches, purity 97.6-99.9 %, stability guaranteed for the duration of the study,	
3.1.2	Specification		
3.1.2.1	Purity		
3.1.2.2	Stability		
3.2	**Test Animals**		
3.2.1	Species	Wistar rats (Strain Bor:WISW (SPF-Cpb); Breeder [redacted])	
3.2.2	Strain		
3.2.3	Source		
3.2.4	Sex	Male / female	
3.2.5	Age/weight at study initiation	68-94 g males / 68-92 g females / 5 weeks old	

Section A6.14/11

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Subchronic toxicity study in rat (administration in drinking water)

3.2.6	Number of animals per group	15 male - 15 female
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	12 weeks
3.3.2	Frequency of exposure	unlimited supply in drinking water
3.3.3	Postexposure period	animals sacrificed at end of exposure period
3.3.4	**Oral**	
3.3.4.1	Type	administered in an unlimited supply of drinking water 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.
3.3.4.2	Concentration	
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.4	**Examinations**	Per OECD 408, no deviations noted by RMS in the December 2005 91/414 draft DAR
3.5	**Sacrifice and pathology**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Appearance, behaviour and mortality of the animals gave no evidence for a treatment-related effect at levels up to 1000 ppm..
4.1.2	Mortality	
4.2	**Body weight gain**	Food intake and body weight development were not affected to a toxicologically significant extent during the entire study
4.3	**Water consumption**	The water intakes were decreased by up to 16 % in the 1000 ppm dose groups.
4.4	**Ophtalmoscopic examination**	Ophthalmic examinations showed no evidence for oculotoxic effects at 1000 ppm.
4.5	**Haematology, urinalysis, clinical chemistry**	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. 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 (see Table A6.14/11-01). The urinalyses and examinations of urinary sediment showed no treatment-related up to and including 1000 ppm.
4.6	**Sacrifice and pathology**	No signs of damage to liver or thyroid were observed at 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.

Section A6.14/11

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Subchronic toxicity study in rat (administration in drinking water)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a subchronic oral toxicity study conducted according to OECD 408 guidelines, imidacloprid nitrosimine was administered to groups of 15 male and 15 female Wistar rats (Strain Bor: WISW, Breeder [redacted]) 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.

5.2 Results and discussion

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.

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. 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. The urinalyses and examinations of urinary sediment showed no treatment-related up to and including 1000 ppm.

Ophthalmic examinations showed no evidence for oculotoxic effects at 1000 ppm.

There were no treatment-related findings noted by gross necropsy, organ weights, histopathology: No signs of damage to liver or thyroid were observed at 1000 ppm. None of the tests produced evidence for an effect on or damage to the kidneys.

5.3 Conclusion

5.3.1	LO(A)EL	300 ppm based on changed haematological parameters
5.3.2	NO(A)EL	100 ppm concentration, equivalent to 13 mg/kg bw/day in drinking water
5.3.3	Other	None
5.3.4	Reliability	1
5.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/01
Materials and Methods	1.1 Test substance: WAK 3839, imidacloprid-nitrosimine
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable:
	NOAEL: 13 mg/kg bw/d, based on changes in haematological parameters at 35/39 mg/kg bw/d (M/F)
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*

Figure A6.14/01: 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

Section A6.14/12

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1/20*
	Authors (year)	[redacted] (1991)
	Title	NTN 38014 - Acute oral toxicity study on rats
	Company, report No.	Bayer CropScience AG, Report-No.: RA91018 BES Ref. : M-028687-01-1
	Date	1991-03-18
	Testing facility	[redacted]
	Dates of work	December 1990 – March 1991
	Test substance(s)	Molecule(s): WAK 4140, imidacloprid Substance(s): NTN 33893 Desnitro
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	NTN 38014 (NTN 33823-desnitro), batch no. TX281190, purity: 87.0 % suspended in polyethylene glycol, stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Description	
3.1.2.2	Purity	
3.1.2.3	Stability	

Section A6.14/12

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

3.2	**Test Animals**	
3.2.1	Species	Sprague Dawley rats (Strain Crj:CD (SPF); Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	Male / female
3.2.5	Age/weight at study initiation	202-219 g for males / 150-171 g for females / 6 weeks old
3.2.6	Number of animals per group	5 male, 5 female
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Postexposure period	14 days
3.3.2	Type	The dosing solutions were administered by stomach tube to fasted Sprague Dawley rats at dose levels of 1000, 630, 390, 240 and 150 mg/kg bw. Application volume: 10 mL/kg bw.
3.3.3	Concentration	
3.3.4	Vehicle	
3.3.5	Concentration in vehicle	
3.3.6	Total volume applied	
3.4	**Examinations**	Clinical signs, gross necropsy
3.5	**Method of determination of LD_{50}**	Method of Thompson
		4 RESULTS AND DISCUSSION
4.1	**Clinical signs**	See Table A6.14/12-1. Sedation, ptosis, abnormal respiration, abnormal gait, tremor, hypothermia of the skin, convulsion, red tear, mortality.
4.2	**Pathology**	Red/brown or gray/whitish patches in lungs; reddening of mucosa in the gastrointestinal tract.
4.3	**LD_{50}**	Males 300 mg/kg bw Females 280 mg/kg bw

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In an acute oral toxicity study conducted according to Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1. guidelines, NTN 33893 desnitro was administered in a single dose by oral gavage to fasted Sprague Dawley rats at dose levels ranging from 150-1000 mg/kg bw.	
5.2	**Results and discussion**	Mortalities occurred at doses at and above 1560 mg/kg bw in males and at and above 240 mg/kg bw in females. Clinical signs included sedation, ptosis, abnormal respiration, abnormal gait, tremor, hypothermia of the skin, convulsion, red tear. Pathology revealed red/brown or gray/whitish patches in lungs; reddening of mucosa in the gastrointestinal tract.	X
5.3	**Conclusion**	LD50 Males 300 mg/kg bw LD50 Females 280 mg/kg bw NTN 33893-desnitro is of moderate toxicity to rats following acute oral administration.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2009/08/24
Materials and Methods	Applicant's version is acceptable.
Results and discussion	5.2 Mortalities occurred at dose levels ≥ 240 mg/kg bw in males and females
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.14/12-1. NTN 33893-desnitro-Acute Oral Toxicity to Rat

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

Section A6.14/13

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /21*	
	Authors (year)	[redacted] (1991c)	
	Title	NTN 38014 - Reverse mutation assay (Salmonella typhimurium and Escherichia coli)	
	Company, report No.	Bayer CropScience AG, Report-No.: RA91019 BES Ref. : M-028689-02-1	
	Date	1991-03-29	
	Testing facility	[redacted]	
	Dates of work	February 1991	
	Test substance(s)	Molecule(s): WAK 4140, imidacloprid Substance(s): NTN 33893 Desnitro	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	NTN 38014 (NTN 33823-desnitro), batch no. TX281190, purity: 87.0 % stability guaranteed for the duration of the study.	
3.1.2	Specification		
3.1.2.1	Purity		
3.1.2.2	Stability		
3.2	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98 E. coli: WP2 uvr A	
3.2.2	Metabolic activation system	S9 mix	
3.2.3	Positive control	AF2, 2-Aminoanthracene, NaN3 and 9-Aminoacridine	

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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, NaN3 and 9-AA were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	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.
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, NTN 33893 desnitro 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 2500 µg per plate. The solvent was DMSO. AF2, 2AA, NaN3 and 9-AA were used as positive controls.
5.2	**Results and discussion**	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.
5.3	**Conclusion**	NTN 33893-desnitro is considered to be non-mutagenic in this assay with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.14/13

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	2.3 Study in accordance without deficiencies to OECD471 (26th May 1983) Deficiencies to OECD471 (21st July 1997): activation activity of S9 solely tested with 2-aminoanthracene
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.14/14

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.8.1, II A, 5.8.1 /03*
	Authors (year)	[redacted] (1991b)
	Title	NTN 35884 - Acute oral toxicity study on rats
	Company, report No.	Bayer CropScience AG, Report-No.: RA91039 BES Ref. : M-028777-01-1
	Date	1991-11-29
	Testing facility	[redacted]
	Dates of work	October – November 1991
	Test substance(s)	Molecule: NTN 35884-olefin metabolite of NTN33893, imidacloprid
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	NTN 35884 (NTN 33893-olefine), batch no. TX221190, purity: 98.0 %. suspended in polyethylene glycol 400. stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Description	
3.1.2.2	Purity	
3.1.2.3	Stability	

Section A6.14/14

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

3.2	**Test Animals**	Sprague Dawley rats (Strain Crj:CD (SPF); Breeder [redacted]), males and females
3.2.1	Species	
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	208-227 g for males / 152-180 g for females / 6 weeks old
3.2.6	Number of animals per group	5 male, 5 female
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	
3.3.1	Postexposure period	14 days
3.3.2	Type	The dosing solutions were administered by stomach tube to 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.
3.3.3	Concentration	
3.3.4	Vehicle	
3.3.5	Concentration in vehicle	
3.3.6	Total volume applied	
3.4	**Examinations**	Clinical signs, gross necropsy
3.5	**Method of determination of LD_{50}**	Method of Thompson

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	See Table A6.14/14-1 for mortality. Clinical signs were mydriasis, abnormal respiration, tremor, lacrimation, chromodacryorrhea, emaciation, abnormal gait, red urine, piloerection.
4.2	**Pathology**	In animals that died: 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.
4.3	**LD_{50}**	Males 3500 mg/kg bw Females 1100 mg/kg bw

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Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted according to Japanese MAFF Guideline No. 3850; OECD 401; FIFRA § 81-1; EEC B.1. guidelines, NTN 33893 olefin was administered in a single dose by oral gavage to fasted Sprague Dawley rats at dose levels ranging from 440-5000 mg/kg bw for males and 200-1500 mg/kg bw for females.
5.2	**Results and discussion**	Mortalities occurred at doses at and above 2200 mg/kg bw in males and at and above 990 mg/kg bw in females. Clinical signs included mydriasis, abnormal respiration, tremor, lacrimation, chromodacryorrhea, emaciation, abnormal gait, red urine, piloerection. Pathology in animals that died revealed 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.
5.3	**Conclusion**	LD50 Males 3500 mg/kg bw LD50 Females 1100 mg/kg bw NTN 33893-olefine is of moderate toxicity to rats following acute oral administration.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable
Conclusion	Applicant's version is acceptable
Reliability	1
Acceptability	Acceptable
Remarks	

Section A6.14/14

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral LD50 study in rat

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.14/14-1. 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

Section A6.14/15

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph B.6.8.1, II A, 5.8.1 /04*
	Authors (year)	[redacted] (1991c)
	Title	NTN 35884 - Reverse mutation assay (Salmonella typhimurium and Escherichia coli)
	Company, report No.	Bayer CropScience AG, Report-No.: RA91040 BES Ref. : M-028781-01-1
	Date	1991-11-29
	Testing facility	[redacted]
	Dates of work	November 1991
	Test substance(s)	Molecule(s): NTN 35884 (NTN 33893-olefine)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	NTN 35884 (NTN 33893-olefine), batch no. TX221190, purity: 98.0 % stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98 E. coli: WP2 uvr A
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	AF2, 2-Aminoanthracene, NaN3 and 9-Aminoacridine

Section A6.14/15

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Gene mutation in Salmonella typhimurium and Escherichia coli

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	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, NaN3 and 9-AA were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	Per Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	NTN 33893-olefine concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. No bacteriotoxicity was observed.
4.1.1	without metabolic activation	
4.1.2	with metabolic activation	
4.2	**Cytotoxicity**	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to Japanese MAFF Guideline No. 4200; OECD 471, 84/449/EEC, FIFRA-PB 84-233295, NTN 33893 olefin 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 2500 µg per plate. The solvent was DMSO. AF2, 2AA, NaN3 and 9-AA were used as positive controls.
5.2	**Results and discussion**	NTN 33823-desnitro concentrations of up to 5000 µg/plate did not produce an increase in the mutant count. Bacterial growth was inhibited at the highest doses with and without metabolic activation.
5.3	**Conclusion**	NTN 33893-olefine is considered to be non-mutagenic the salmonella/microsome test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/12
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

This document has been prepared by the competent authority and does not necessarily represent the participant's opinion.

Section A6.14/16
Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance
Acute oral toxicity test in rats

Official use only

1 REFERENCE

1.1 Reference

[redacted] (1991): NTN 33519 – Acute oral toxicity study on rats. [redacted] unpublished report No.: RA91023, date: May 31, 1991.

1.2 Data protection

Yes

1.2.1 Data owner

Submitted for inclusion into annex I of Dir. 91/414/EEC by Bayer Crop Science

1.2.2 Criteria for data protection

Unpublished report

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

Japanese MAFF Guideline; OECD 401; FIFRA § 81-1; EEC B.1

2.2 GLP

Yes (certified laboratory)

2.3 Deviations

None

3 MATERIALS AND METHODS

Test material
NTN 33519 (NTN33893-urea), batch no. TX040391, purity: 99.9 %

Test animals
Sprague Dawley rats (Strain Crj:CD (SPF); Breeder [redacted])

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.

4 RESULTS AND DISCUSSION

Mortality
Cf. CA-Table 1 (Appendix to this summary)

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

This document has been prepared by the competent authority and does not necessarily represent the participant's opinion.

Section A6.14/16

Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance

Acute oral toxicity test in rats

5 AUTHORITY'S SUMMARY AND CONCLUSION

5.1 Conclusion

NTN 33893-urea is of moderate toxicity to rats following acute oral administration. An LD_{50} of 4000/1820 mg/kg bw (M/F) was calculated proving that this metabolite is of lower acute oral toxicity than the parent compound.

5.2 Reliability

1

Acceptable without restrictions

5.2.1 Deficiencies

None relevant

Appendix: CA-Table

CA-Table 1: 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

This document has been prepared by the competent authority and does not necessarily represent the participant's opinion.

Section A6.14/17
Annex Point IIIAXI.2

Toxic effects of substances generated from an active substance
Gene mutation in Salmonella typhimurium and Escherichia coli

Official use only

1 REFERENCE

1.1 Reference

[redacted] (1991): NTN 33519 – Reverse mutation assay (Salmonella typhimurium and Escherichia coli).

[redacted]., unpublished report No.: RA91024, date: July 22, 1991.

1.2 Data protection

Yes

1.2.1 Data owner

Submitted for inclusion into annex I of Dir. 91/414/EEC by Bayer Crop Science

1.2.2 Criteria for data protection

Unpublished report

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

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

2.2 GLP

Yes (certified laboratory)

2.3 Deviations

None

3 MATERIALS AND METHODS

Test material

NTN 33519 (NTN33893-urea), batch no. TX040391, purity: 99.9 %

Test strains

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, NaN3 and 9-AA were used as positive controls.

4 RESULTS AND DISCUSSION

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

5 AUTHORITY'S SUMMARY AND CONCLUSION

5.1 Conclusion

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

5.2 Reliability

1

Acceptable without restrictions

5.2.1 Deficiencies

None relevant

Section A6.15.3/01

Annex Point IIIA, XI.1.4

Estimation of exposure to humans or animals through food and feeding stuffs and other means

Metabolism study in the lactating goat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.7.2.1, II A, 6.2.2.1 /01*
	Authors (year)	[redacted] (1991)
	Title	(Pyridinyl-^{14}C-methylene) imidacloprid: Absorption, distribution, excretion and metabolism in a lactating goat
	Company, report No.	Bayer CropScience AG, Report-No.: PF3731 BES Ref. : M-024212-01-1
	Date	1991-12-18
	Testing facility	[redacted]
	Dates of work	September 13, 1988 to October 29, 1991
	Test substance(s)	Molecule(s): imidacloprid Purity 99.5% Substance(s): [pyridinyl-^{14}C-methylene] NTN 33893 labelled Specific radioactivity 3.2 MBq/mg, radiochemical purity >99%
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

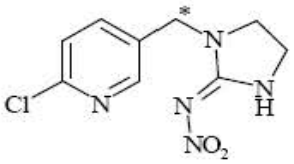
2.1	**Guideline study**	EPA Pesticide Assessment Guidelines Subdivision O, Residue Chemistry, Series 171-4: Nature of the Residue, Livestock (Ruminant) EPA 540/9-82-023, October, 1982
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	[pyridinyl-^{14}C-methylene] NTN 33893 labelled
3.1.1	Lot/Batch number	^{14}C -Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 3.2 MBq/mg, radiochemical purity > 99 %
3.1.2	Specification	

Section A6.15.3/01
Annex Point IIIA, XI.1.4

Estimation of exposure to humans or animals through food and feeding stuffs and other means
Metabolism study in the lactating goat

3.1.2.1	Purity	
3.1.2.2	Stability	Stable for the duration of the study
3.1.2.3	Radiolabelling	
3.1.2.4	Reference standards	See Table A6.15.3/01-1
3.2	**Test Animals**	
3.2.1	Species	Bunte deutsche Edelziege, lactating, *(Capra hircus)*
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	about 18 months, weight range 29.7-31 kg
3.2.6	Number of animals per group	1
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Concentration of test substance	10 mg/mlin aqueous traganth (0.5%)
3.3.2	Specific activity of test substance	Diluted specificity 0.322 MBq/mg
3.3.3	Volume applied	10 mg/kg bw
3.3.4	Exposure period	3 days
3.3.5	Sampling time	See Table A6.15.3/01-2 for excreta times; tissues and organs at 50h

Section A6.15.3/01

Annex Point IIIA, XI.1.4

Estimation of exposure to humans or animals through food and feeding stuffs and other means

Metabolism study in the lactating goat

3.3.6	Samples	**Blood:** Micro-samples of blood (about 50 µL) were taken from the ear veins of the goat at 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after the first administration. The plasma was separated by centrifugation weighed and radioassayed. **Milk:** The goat was milked in the morning immediately prior to each dosing, ca. 8 hours later and immediately before sacrifice. Milk volumes were recorded, aliquots were taken and radioassayed and the remaining portion was stored at -20 °C for analysis. **Urine:** The urine fractions were collected as quantitatively as possible with dry ice cooling at 8 and 24 hours after each administration, immediately before the next dosage the collection vessel was changed. After recording the total volume radioactivity was determined in subsamples by LSC. The remaining urine was stored for an optional analysis at -20 °C. **Faeces:** The faeces were collected as quantitatively as possible immediately before the next dosage. The faeces fractions were freeze-dried and homogenised. After recording the total dry weight the radioactivity was determined by combustion and LSC of combustion gases. The remaining material was stored for an optional analysis at -20 °C. **Organs / tissues:** The following tissues and organs were dissected: Liver, kidney, three different types of muscles (loin, round, flank) and three different types of fat (perirenal, subcutaneous, omental). After recording the weights the samples were transferred into ice-cooled vessels. Liver, kidney and muscle samples homogenised. Subsamples were freeze-dried (absence of volatile radioactive components had been checked) and radioassayed by combustion and LSC of combustion gases. Fat samples were solubilised without homogenisation for radioactivity measurement by LSC.

Section A6.15.3/01

Annex Point IIIA, XI.1.4

Estimation of exposure to humans or animals through food and feeding stuffs and other means

Metabolism study in the lactating goat

3.3.7	Metabolite isolation and purification	**Urine** collected after the second administration was separated by prep. RP-HPLC into eight fractions. The lyophilised fractions were further fractionated by analytical scale RP-HPLC. Eluates appearing to contain one major component were subjected to H-NMR and/or MS spectroscopy. One fraction was further purified by prep. TLC and then analysed by H-NMR. One fraction was analysed after methylation by GC-MS. **Milk** was centrifuged for separation of the coagulated milk protein. The supernatant was concentrated by lyophilisation. Separation into fractions for identification of metabolites was performed using partitioning, micro-prep. RP-HPLC and TLC. **Liver** tissue was minced and exhaustively extracted with water. The extraction residues were lyophilised and radioassayed by combustion followed by LSC of combustion gases. The water extracts were charged onto a chromatography column filled with adsorber resin which had been pre- conditioned with methanol-water. It was eluted firstly with water, then with methanol. This procedure yielded a water and methanol phase. The water phase was adjusted to pH 2 with heptanesulphonic acid–citric acid. The precipitated protein was removed by centrifugation and the supernatant was subjected to further purification by adsorber resin chromatography using water in a first step followed by methanol. The methanol phase was further purified using RP18-silica gel in the batch mode. The radioactivity was successively eluted with methanol, acetonitrile and 1 % acetic acid followed by a rinse with acetonitrile. The first two organic phases which contained the majority of the radioactivity were combined and concentrated. The resulting phases ultimately representing the methanol and the water phases from the first step were finally purified on silica gel cartridges using a sequence of polar mixtures of organic solvent. The solvent fractions were then separately subjected to RP-HPLC in order to identify and quantify metabolites. **Kidney** homogenate was extracted twice with mixtures of methanol and ethyl acetate. The combined extracts were concentrated and radioassayed. A portion was concentrated to near dryness, redissolved in water and subjected to prep. RP-HPLC. The fractions obtained were characterized and quantified by several TLC systems. **Muscle** homogenate was suspended in water under cooling using an Ultra-Turrax homogeniser. After centrifugation the sediment was re-extracted with water three times. The combined extracts were concentrated by lyophilisation and mixed with acetonitrile. The emulsion formed was separated into two phases. The lower phase was re-extracted several times with acetonitrile and the combined upper phases were concentrated. An aliquot was concentrated and subjected to micro-preparative scale RP-HPLC. This procedure yielded a number of peaks, which were further characterised by TLC. **Fat** homogenate was suspended in acetonitrile using an Ultra-Turrax under cooling. After centrifugation the sediment was re-extracted and the combined extracts were concentrated. For further separation an aliquot was concentrated and subjected to micro-preparative scale RP-HPLC. This procedure yielded a number of peaks, which were further characterised by TLC.

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3.3.8	Metabolite quantitation and identification	**Analysis and structure elucidation:** RP-HPLC with UV-spectrophotometer and flow-through radioactivity detector. TLC using radioactivity scanning with an linear analyser. Identification of peak components using reference substances with co-chromatography Mass spectroscopy in direct inlet mode, in some cases GC-MS H-NMR, 360 MHz and 500 MHz Samples subjected to GC-MS were derivatised into the methylated compound with diazomethane. Enzymatic cleavage with ß-glucuronidase/aryl sulfatase **Measurement of radioactivity:** Measurement of solid samples using LSC: 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 added 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) are calculated from relative concentrations by multiplying with the dose in mg per kg. Amounts of radioactivity present in the excrets or still present at time of sacrifice in the tissues of the animal body or in the organs are calculated from measured concentrations and the weight contribution to the total body weight.

4 RESULTS AND DISCUSSION

4.1 Absorption and excretion

The recovery of radioactivity and the excretion pattern after a triple oral administration of 10 mg per day is shown in Table A6.15.3/01-2. Until sacrifice (50 hours after the first administration) the excretion amounted to 49.6 % of the administered radioactivity. The excretion with the urine was the predominant route of elimination. By this route 39.7 % of the total dose were eliminated. The renal excretion rate was very high. About one third of the quantity of radioactivity eliminated in total from the body in the test period was excreted within the first 8 hours after each administration. The faecal excretion was low with a value of 9.6 %. A very low amount (0.23 %) was excreted with the milk. From the predominant renal excretion of radioactivity it can be concluded that the compound-related radioactivity was absorbed to a high extent.

The level of radioactivity in the plasma reached the maximum at 2 hours after the first administration with the equivalent concentration of 3.98 µg/mL, corresponding to about 40 % of the equidistribution in the body (Table A6.15.3/01-3 and Figure A6.15.3/01-1). The absorption rate was fast as shown by the characteristic ta = 0.48 hours (representing the time elapsed for an increase in concentration from 25 % to 75 % of the maximum value) and by the plasma peak level which was reached shortly after the first administration. The radioactivity was eliminated from the plasma with a half-life of about 4.8 hours for the time period from 2 to 24 hours after the first administration.

Comparable equivalent concentrations in milk were measured 8 hours after the first and after the second administration. Within the next 16 hours after the 1st and 2nd administration the concentration in the milk was reduced by comparable factors of 12 and 11, respectively. The highest equivalent concentration of 4.1 µg/g was determined 2 hours after the third application. In total 0.23 % of the total administered dose was found in milk. The detailed results are given in Table A6.15.3/01-4.

The radioactivity levels measured in the samples of tissues and organs as well as the respective weights are given in Table A6.15.3/01-5.The equivalent concentrations in the three different types of muscle ranged from 3.80 to 3.96 µg/g. The total residues in the body musculature amounted to 3.45 % of the radioactivity totally administered, assuming that the musculature accounted for 30 % of body weight. Even lower quantities were determined in the different fat tissues. The mean value in fat tissues was 2.40 µg/g which corresponds to 0.73 % of the totally administered radioactivity. It was assumed that the fat tissues accounted for 12 % of the body weight. The highest equivalent concentration was 15.92 µg/g determined in the liver followed by 11.59 µg/g in kidney. This result reflects the significance of these organs for metabolism and excretion of the test compound and its labelled biotransformation products.

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4.2 Metabolism

The radioactive residues were extracted from milk with acetonitrile or with methanol and acetonitrile. In highly radioactive milk samples, after separating the milk fat and the coagulated milk protein the extraction of the remaining aqueous whey with acetonitrile yielded 85 to 92 % of the initial radioactivity indicating the lack of protein bound residues. In milk samples of low levels of radioactivity 68 and 72 % of the initial radioactivity were determined. The balance of the radioactivity extraction from kidney, liver, muscle and fat is given as percent of initial radioactivity in Table A6.15.3/01-6.

In **milk** samples collected at peak concentrations 8 hours after the 1st and 2nd administration and 2 hours after the 3rd dose about 80 % of the total recovered radioactivity were identified. See Table A6.15.3/01-7 for metabolite profile and levels.

In **kidney** only 37.7 % of the total recovered radioactivity was identified (see Table A6.15.3/01-7 for levels and profile).

In **liver** the identification rate was low (14.38 % of the total recovered radioactivity). The reason for this low rate is at least twofold. At first a large amount of matrix components were co-extracted with the radioactivity because sufficient extraction rates could be obtained only with water. Furthermore, due to the reactive structures of the identified imino- and guanidino-type metabolites it can be assumed that these metabolites possessed a high affinity to the coextracted matrix components. Therefore they could not be isolated and purified for the identification process. See Table A6.15.3/01-8 for levels and profile.

The metabolites of the three different **muscle** types, round, flank and loin, were purified and identified separately. In total about 78 to 87 % of the total recovered radioactivity was identified. . Table A6.15.3/01-9 shows that the metabolism of imidacloprid in the three different muscle types does neither differ in qualitative nor in quantitative terms.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a study conducted according to EPA guideline 171-4, the absorption, distribution, excretion and metabolism of imidacloprid in lactating goat was elucidated after three consecutive daily administrations of radiolabelled test substance administered by intubation of an aqueous solution once daily at a target dose level of 10 mg/kg bw. The treated animal was sacrificed at plasma peak level as determined after the first administration. The untreated companion goat was sacrificed the day before.

Samples of plasma, milk, urine and faeces were taken at pre-determined times; organs (liver, kidney) and tissues (muscles and fats) were taken after sacrifice on day 3.

Samples were radioassayed, metabolites extracted, purified and identified using appropriate analytical techniques.

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5.2 Results and discussion

Until sacrifice (50 hours after the first administration) excretion amounted to 49.6 % of the administered radioactivity. The excretion with the urine was the predominant route of elimination. By this route 39.7 % of the total dose were eliminated. The renal excretion rate was very high. About one third of the quantity of radioactivity eliminated in total from the body in the test period was excreted within the first 8 hours after each administration. The faecal excretion was low with a value of 9.6 %. From the predominant renal excretion of radioactivity it can be concluded that the compound-related radioactivity was absorbed to a high extent.

Comparable equivalent concentrations in milk were measured 8 hours after the first and after the second administration. Within the next 16 hours after the 1st and 2nd administration the concentration in the milk was reduced by comparable factors of 12 and 11, respectively. The highest equivalent concentration of 4.1 µg/g was determined 2 hours after the third application. In total 0.23 % of the total administered dose was found in milk.

The equivalent concentrations in the three different types of muscle ranged from 3.80 to 3.96 µg/g. The total residues in the body musculature amounted to 3.45 % of the radioactivity totally administered, assuming that the musculature accounted for 30 % of body weight. Even lower quantities were determined in the different fat tissues. The mean value in fat tissues was 2.40 µg/g which corresponds to 0.73 % of the totally administered radioactivity. It was assumed that the fat tissues accounted for 12 % of the body weight. The highest equivalent concentration was 15.92 µg/g determined in the liver followed by 11.59 µg/g in kidney. This result reflects the significance of these organs for metabolism and excretion of the test compound and its labelled biotransformation products.

In milk, the main component was unchanged imidacloprid, accounting for 41 to 55 % of the total recovered radioactivity. Imidacloprid-5-hydroxy (M01) and imidacloprid-4-hydroxy (M02) and their common dehydration product imidacloprid-olefine (M06) were detected as minor comppnents. In milk samples collected 24 hrs. after only 27 % of the total recovered radioactivity could be identified due to low concentrations of residues and the complexity of the matrix. Besides the parent imidacloprid- 4-hydroxy (M02), imidacloprid-5-hydroxy (M01) and imidacloprid-olefine (M06) were identified and quantified. The metabolites imidacloprid-6-CNA-glycine (M15) and imidacloprid-nitrosimine (M07) were only found in traces in milk samples.

In kidney, metabolite imidacloprid-6-CNA-glycine (M15) accounted for about 13 % of the total recovered radioactivity (additional efforts are reported in A6.15.3/02). Imidacloprid was of minor importance with about 6 %. As minor metabolites, imidacloprid-5- hydroxy (M01) and imidacloprid-4-hydroxy (M02), imidacloprid-olefine (M06) and imidacloprid-5- hydroxy-glucoronide (M04) were identified. imidacloprid-nitrosimine (M07) was detected in traces.

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5.2	**continued**	In liver the identification rate was also low (14.38 % of the total recovered radioactivity). The reason for this low rate is at least twofold. At first a large amount of matrix components were co-extracted with the radioactivity because sufficient extraction rates could be obtained only with water. Furthermore, due to the reactive structures of the identified imino- and guanidino-type metabolites it can be assumed that these metabolites possessed a high affinity to the coextracted matrix components. Therefore they could not be isolated and purified for the identification process. Additional efforts are reported in A6.15.3/02. As a result the main metabolites identified are the imino-type metabolites imidacloprid-ring-open-guanidine (M10) and/or imidacloprid-desnitro (M09). As minor components were identified imidacloprid (0.13 %), imidacloprid-6-CNA-glycine (M15) (1.8 %), 6-Cl-nicotinic acid (M14) (1.5 %), imidacloprid- PEDA (M22) (0.2 %) and imidacloprid-urea (M12) (0.04 %). The metabolites of the three different muscle types, round, flank and loin, were purified and identified separately. In total about 78 to 87 % of the total recovered radioactivity was identified. The main component was imidacloprid, accounting for 64 to 69 % of the total recovered radioactivity. As minor metabolites imidacloprid-5-hydroxy (M01), imidacloprid-4-hydroxy (M02), imidacloprid-5-hydroxy-glucoronide (M04) and imidacloprid-olefine (M06) were identified. Only traces of imidacloprid-nitrosimine (M07) were present. Metabolism of imidacloprid in the three different muscle types does neither differ in qualitative nor in quantitative terms.
5.3	**Conclusion**	The overall conclusion of ruminant metabolism can be found in the conclusion section of A6.15.3/02.
5.3.1	Reliability	1
5.3.2	Deficiencies	No, with the understanding further efforts on elucidating metabolism in liver and kidney are reported in study summary A6.15.3/02