concentration was lowered from 1800 down to 1200 ppm from wk 5 on. Starting in wk 6, tremor and/or trembling were no longer observed until the end of the study. Unfortunately test compound intake was reported only on a weekly basis in the 90-d study report. Before administration the food was mashed (by mixing powdered food/test item mix 1:1 with water)

Tremor at 1800/1200 ppm

When averaged over the whole study period of 90 days this feed concentration corresponded to an mean group dose level of 45.4 mg/kg bw/d. (Severe) tremor in this group, however, was only described during wk 1 and trembling occurred only up to wk 5, but did not continue to be observed when feed concentration had been lowered to 1200 ppm.

Conclusions

- In the mid-dose group at 600 ppm, only single or double transient incidences of trembling were observed, presumably on one of the first days of dosing.
- The wording of "trembling" vs. "(severe) tremor" suggests a mild intensity. Furthermore, after week 1 some sort of adaptation/tolerance seems to have developed, as Because trembling was not reported for this group during weeks 2-13.
- Tremor or trembling in the high-dose group were only observed at dose levels believed to be clearly above the 90-d group mean dose level of 45.4 mg/kg bw/d.

Additional 14-d comparative pilot study on food consumption

An additional study was performed by one of the authors of the 90-d study in order to examine the influence of pelleted vs. mashed food on the study outcome. A total of 8 animals (2 M + 2 F per group) received 1200 mg Imidacloprid/kg feed either in mashed or pelleted form. Feed and thereby substance intake was higher with pelleted than with mashed feed.

More notably, trembling/tremor was not observed in any of the groups.

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Section A6.4.1.2/02 Subchronic Oral Toxicity Test

Annex Point IIA6.4

52 Week Dietary Administration to Dog

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1 REFERENCE

1.1	**Reference**	*PPP Monograph B6.3.2.3, II A, 5.5.3 /01*
	Authors (year)	[redacted] (1989)
	Title	52-week oral toxicity (feeding) study with NTN 33893 technical in the dog
	Company, report No.	Bayer CropScience AG, Report-No.: R4856 BES Ref. : M-027093-02-1
	Date	1989-10-19, Amended: 1992-03-03
	Testing facility	[redacted]
	Dates of work	October 1987 – October 1988
	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 452; FIFRA § 83-1
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity 94.9 %.
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	Beagle dog (Breeder [redacted]
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	

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Section A6.4.1.2/02 Subchronic Oral Toxicity Test

Annex Point IIA6.4

52 Week Dietary Administration to Dog

3.2.5	Age/weight at study initiation	6.6 - 9.2 kg males - 5.3 - 7.4 kg females - 4 to 6 months old
3.2.6	Number of animals per group	4 male and 4 female
3.2.7	Control animals	yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	52 weeks
3.3.2	Frequency of exposure	Daily in diet
3.3.3	Postexposure period	All animals sacrificed at end of exposure period
3.3.4	**Oral**	
3.3.4.1	Type	In diet at concentrations of 0, 200, 500 or 1250/2500 ppm. The concentration of 1250 ppm was increased to 2500 ppm from treatment week 17 onwards. Mean uptakes of imidacloprid were 0, 6.1, 15 or 41/72 mg/kg bw/day for males and females combined.
3.3.4.2	Concentration	
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.3.4.5	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	Per OECD 452; FIFRA § 83-1, no deviations noted by RMS in the December 2005 91/414 draft DAR
3.4.1.1	Clinical signs	
3.4.1.2	Mortality	
3.4.2	Body weight	
3.4.3	Food consumption	
3.4.4	Water consumption	
3.4.5	Ophthalmoscopic examination	
3.4.6	Haematology	
3.4.7	Clinical Chemisty	
3.4.8	Urinalysis	
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Per OECD 452; FIFRA § 83-1, no deviations noted by RMS in the December 2005 91/414 draft DAR
3.5.2	Gross and histopathology	
3.5.3	Other examinations	none

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Section A6.4.1.2/02 **Subchronic Oral Toxicity Test**

Annex Point IIA6.4 *52 Week Dietary Administration to Dog*

3.5.4	Statistics	ANOVA, Dunnett's t-test
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Appearance, behaviour, and mortality were unaffected in males and females at 1250/2500 ppm.
4.1.2	Mortality	In contrast to the findings of the 90-day study no indications of trembling or tremor were observed which supports a NOAEL for this endpoint of 40-50 mg/kg bw/day.
4.2	**Body weight gain**	Body weight gains were unaffected in males and females at 1250/2500 ppm.
4.3	**Food consumption and compound intake**	Initial slight reductions in food intake were observed in both sexes at 1250 ppm and when the concentration was increased to 2500 ppm..
4.4	**Ophtalmoscopic examination**	Ophthalmic examinations and hearing tests indicated no treatment-related changes at 1250/2500 ppm doses
4.5	**Blood analysis**	
4.5.1	Haematology	At 1250/2500 ppm a slight increase in the plasma cholesterol levels in the females and slight increases in the hepatic cytochrome P-450 values in males and females were observed. (see Table 6.4.1.2/02-1).
4.5.2	Clinical Chemisty	
4.5.3	Urinalysis	
4.6	**Sacrifice and pathology**	
4.6.1	Organ Weights	Slightly elevated liver weights which were observed at 1250/2500 ppm (see Table 6.4.1.2/02-1) can be seen as an adaptation process of the organ for the metabolisation of imidacloprid. The gross pathology and histopathology produced no evidence for treatment-related changes in the organs and tissues examined.
4.6.2	Gross and histopathology	
4.7	**Other**	Due to the fact that enzyme induction was observed in the high dose group the increase of the test substance concentration in the food did not necessarily result in much higher plasma levels of the active substance after week 17.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In a subchronic oral toxicity study conducted according to OECD 452; FIFRA § 83-1 guidelines, groups of 4 male and 4 female pure-bred beagle dogs were administered 94.9% pure imidacloprid technical at concentrations of 0, 200, 500 or 1250/2500 ppm in their diet for a period of 52 weeks. The concentration of 1250 ppm was increased to 2500 ppm from treatment week 17 onwards. Mean uptakes of imidacloprid were 0, 6.1, 15 or 41/72 mg/kg bw/day for males and females combined.

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Section A6.4.1.2/02 Subchronic Oral Toxicity Test

Annex Point IIA6.4

52 Week Dietary Administration to Dog

5.2	**Results and discussion**	Appearance, behaviour, body weight gains and mortality were unaffected in males and females at 1250/2500 ppm. Initial slight reductions in food intake were observed in both sexes at 1250 ppm and when the concentration was increased to 2500 ppm. Ophthalmic examinations and hearing tests indicated no treatment-related changes at 1250/2500 ppm doses. In contrast to the findings of the 90-day study no indications of trembling or tremor were observed which supports a NOAEL for this endpoint of 40-50 mg/kg bw/day. Due to the fact that enzyme induction was observed in the high dose group the increase of the test substance concentration in the food did not necessarily result in much higher plasma levels of the active substance after week 17. There were no treatment-related effects on haematology test parameters up to a dose of 1250/2500 ppm. At 1250/2500 ppm a slight increase in the plasma cholesterol levels in the females and slight increases in the hepatic cytochrome P-450 values in males and females were observed. No treatment-related effects were seen on urinary parameters. Slightly elevated liver weights which were observed at 1250/2500 ppm can be seen as an adaptation process of the organ for the metabolisation of imidacloprid. The gross pathology and histopathology produced no evidence for treatment-related changes in the organs and tissues examined. No further organ weight changes occurred.
5.3	**Conclusion**	
5.3.1	LO(A)EL	1250/2500 ppm, equivalent to 41/72 mg/kg bw/day.
5.3.2	NO(A)EL	NOAEL: 500 ppm, equivalent to 15 mg/kg bw/day based on liver effects (slight increase of weight, plasma cholesterol and cytochrome P-450) at 1250/2500 ppm.
5.3.3	Reliability	1
5.3.4	Deficiencies	None

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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	Applicant's version is basically acceptable. However, in response to questions/comments of the other MS during the peer review process, the RMS provided an additional document, in which a. o. the findings in the available repeat-dose dog studies were analysed in more detail. The corresponding section from this document referring to the 1-yr dog study is reproduced here: *" [...] In this guideline-conform, GLP study of reliability 1, trembling/tremor was not observed at dose levels of 200, 500, or 1250/2500 mg Imidacloprid /kg (pelleted) feed, equivalent to group mean doses of 6.1, 15, and 41/72 mg/kg bw/d. The dose level of the high-dose group was raised from 1250 to 2500 ppm from wk 17 on. Perhaps because enzyme induction had taken place by that time-point, subsequent plasma peak levels might have been lower than would be expected if 2500 ppm had been administered right from the start.* *Treatment with 1250 ppm was associated with a slight (below or in the order of 10 %) and transient fall in food consumption in males (week 1) and females (weeks 1 and 2). A similar transient effect was seen when the dietary concentration was increased in week 17, the effects being seen in males in weeks 17-18 and in females in weeks 17-20. Overall body weight gain was not affected in a dose-related fashion."*
Conclusion	Applicant's version is agreed in that the observed effects with regard to their nature and extent have to be considered common adaptation processes and do not constitute adverse effects. As a consequence, the following NOAEL/LOAEL values are derived: LOAEL: > 41 mg/kg bw/d (1250/2500 ppm) NOAEL: 41 mg/kg bw/d (500 ppm)
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	

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Table A6.4.1.2/02-1: 1-year study on dogs – Clinical chemistry and liver weights

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

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

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Section A6.5/01 Chronic toxicity

Section A6.5/02

Chronic toxicity and carcinogenicity study in the rat

Annex Point IIA6.5

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1 REFERENCE

1.1	**Reference**	*PPP Monograph B6.5.1, II A, 5.5.1 /01and 02*	
	Authors (year)	[redacted] (1991 a and b)	
	Title	a) NTN 33893 (proposed c.n.: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months) b) NTN 33893 (proposed common name: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months) - supplementary MTD study for two-year study T1025699	
	Company, report No.	Bayer CropScience AG, Report-No.: a) 19925 b) 20541 BES Ref. : a) M-027741-02-1 b)M-027135-01-1	
	Date	a) 1991-01-25 b) 1991-08-19	
	Testing facility	[redacted]	
	Dates of work	a) July 1987 – July 1989 b) September 1988 – September 1990	
	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			
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 453, FIFRA § 83-5, EU 88/302/EEC	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	No	X

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid, mixed batch no.180587, purity: 94.3 % - 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		

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Section A6.5/01 Chronic toxicity

Section A6.5/02 *Chronic toxicity and carcinogenicity study in the rat*

Annex Point IIA6.5

3.2	**Test Animals**	
3.2.1	Species	Male and female Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder [redacted]
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	56-102 g males / 59-89 g females / 4-5 weeks old
3.2.6	Number of animals per group	50 m, 50f/dose group in main and supplemental study 10m, 10f/dose group as 12 month sacrifice in both studies
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	2 years
3.3.2	Frequency of exposure	Continual via diet
3.3.3	Postexposure period	All animals sacrificed at end of exposure period
3.3.4	**Oral**	
3.3.4.1	Type	Main study in diet at concentrations of 0, 100, 300 and 900 ppm for 24 months. In a supplement MTD study, groups of 50 male and female Wistar rats were administered imidacloprid at levels of 0 and 1800 ppm in their diet for 24 months. Mean consumption of imidacloprid per kg body weight per day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.
3.3.4.2	Concentration	
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	Yes, per OECD 453, FIFRA § 83-5, EU 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.4.1.1	Clinical signs	
3.4.1.2	Mortality	
3.4.2	Body weight	
3.4.3	Food consumption	
3.4.4	Water consumption	
3.4.5	Ophthalmoscopic examination	
3.4.6	Haematology	

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Section A6.5/01 **Chronic toxicity**

Section A6.5/02 *Chronic toxicity and carcinogenicity study in the rat*

Annex Point IIA6.5

3.4.7	Urinalysis		
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, per OECD 453, FIFRA § 83-5, EU 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR	
3.5.2	Gross and histopathology		
3.5.3	Other examinations	TSH, T3 and T4 levels	
3.5.4	Statistics	Mann-Whitney + Wilcoxon 2-tailed U-test, variance analyses	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Appearance, behaviour, food intakes and mortality were unaffected in males and females at 1800 ppm. Water intake was reduced by 13 % in females at 1800 ppm.	
4.1.2	Mortality		
4.2	**Body weight gain**	See Figure A6.5/01 & /02-1 to Figure A6.5/01 & /02-4. Reduced weight gains were noted in males and females at 900 ppm and above with the decreases amounting to 11 – 12 % at 1800 ppm. The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight.	X
4.3	**Food consumption and compound intake**	No treatment related effects on food consumption noted. Mean consumption of imidacloprid per kg body weight and day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.	
4.4	**Ophtalmoscopic examination**	No treatment related effects noted.	
4.5	**Blood analysis**		
4.5.1	Haematology	The haematological tests gave no indications of haematotoxicity or damage to the haematogenic organs at dose levels up to 1800 ppm. The plasma, erythrocyte and brain cholinesterase activities were not significantly affected; adverse effects on, or functional impairment of, any organ could not detected in males and females up to and including 1800 ppm.	
4.5.2	Clinical chemistry		
4.5.3	Urinalysis		
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Absolute liver and kidney weights were reduced after 12 months in	X

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4.6.2	Gross and histopathology	females at 900 ppm; males exhibited lower liver weights at 900 ppm at this time. These deviations from the control values are not attributed to liver or kidney damage, but are seen in relation to the reduced body weight gain in these dose groups (see Table A6.5/01 & /02-1). Histopathological assessment of these organs produced no evidence for treatment-related lesions (see Table A6.5/01 & /02-02). Increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in males beginning at 300 ppm and in females at 900 ppm. At 1800 ppm fewer colloid aggregations and more parafollicular hyperplasias of minimal intensity were observed. These findings occur spontaneously in ageing rats and indicate involution of isolated follicles related to senescence. In these studies they are regarded as a treatment effect on the thyroid resulting in premature biological ageing processes in this organ.	
4.7	**Other**	No treatment related changes in TSH, T3 or T4 were observed after 76 weeks of treatment at 1800 ppm.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a combined chronic toxicity oncogenicity study done according to OECD 453, FIFRA § 83-5, EU 88/302/EEC guidelines, imidacloprid was administered to groups of 50 male and 50 female Wistar rats in their diet at concentrations of 0, 100, 300 and 900 ppm for 24 months. In a supplement MTD study, groups of 50 male and female Wistar rats were administered imidacloprid at levels of 0 and 1800 ppm in their diet for 24 months. Ten additional rats per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.	
5.2	**Results and discussion**	Appearance, behaviour, food intakes and mortality were unaffected in males and females at 1800 ppm. Water intake was reduced by 13 % in females at 1800 ppm. Reduced weight gains were noted in males and females at 900 ppm and above with the decreases amounting to 11 – 12 % at 1800 ppm. The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight.	X
		The haematological tests gave no indications of haematotoxicity or damage to the haematogenic organs at dose levels up to 1800 ppm. The plasma, erythrocyte and brain cholinesterase activities were not significantly affected; adverse effects on, or functional impairment of any organ could not detected in males and females up to and including 1800 ppm.	
		Absolute liver and kidney weights were reduced after 12 months in females at 900 ppm; males exhibited lower liver weights at 900 ppm at this time. These deviations from the control values are not attributed to liver or kidney damage, but are seen in relation to the reduced body weight gain in these dose groups.	X

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		Histopathological assessment of these organs produced no evidence for treatment-related lesions. Increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in males beginning at 300 ppm and in females at 900 ppm. At 1800 ppm fewer colloid aggregations and more parafollicular hyperplasias of minimal intensity were observed. These findings occur spontaneously in ageing rats and indicate involution of isolated follicles related to senescence. In these studies they are regarded as a treatment effect on the thyroid resulting in premature biological ageing processes in this organ. No treatment related changes in TSH, T3 or T4 were observed after 76 weeks of treatment at 1800 ppm. The nature, location, incidence and latency periods of the tumors in this study presented no evidence for an oncogenic effect of imidacloprid.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	300 ppm and 900 ppm in males and females, respectively , based on thyroid effects (increased incidence of colloid mineralisation) and on reduced body weight gains at 900 ppm in both sexes	
5.3.2	NO(A)EL	100/300 ppm (males/females), equivalent of 5.7 mg/kg bw/day for males and 24.9 mg/kg bw/day for females	
5.3.3	Other	The nature, location, incidence and latency periods of the tumors in this study presented no evidence for an oncogenic effect of imidacloprid.	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	X

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/05
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.2/5.2 Reduced weight gain was noted in females at 1800 ppm (-12 %). The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in body weight of more than 10 % (cf. CA-Table 1). 4.6.1/5.2 Absolute liver, kidney, adrenal, heart, and spleen weights were significantly reduced after 24 months in females at 1800 ppm; males exhibited lower liver weights at 900 ppm after 12 months. Except for the liver weight at 1800 ppm in females after 24 months, relative organ weights do not differ statistically from the control values. Thus, the reduced organ weights are considered not to be attributed to organ damage, but are seen in relation to the reduced body weight gain in these dose groups at the specified times (see Table A6.5/01 & /02-1, CA-Table 2).
Conclusion	Applicant's version is acceptable: LOAEL: 17/73 mg/kg bw/d (M/F) based on mineralisation in the colloid of the thyroid gland follicles. NOAEL: 6/25 mg/kg bw/d (M/F)
Reliability	1
Acceptability	Acceptable
Remarks	2.3/5.3.5 Deficiencies: 10 instead of 20 animals in the satellite groups for interim sacrifice. The deviation does not affect the overall validity of the study.
	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	

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Figure A6.5/01 & /02-1: Main chronic study in rats - Body weights (mean values) males

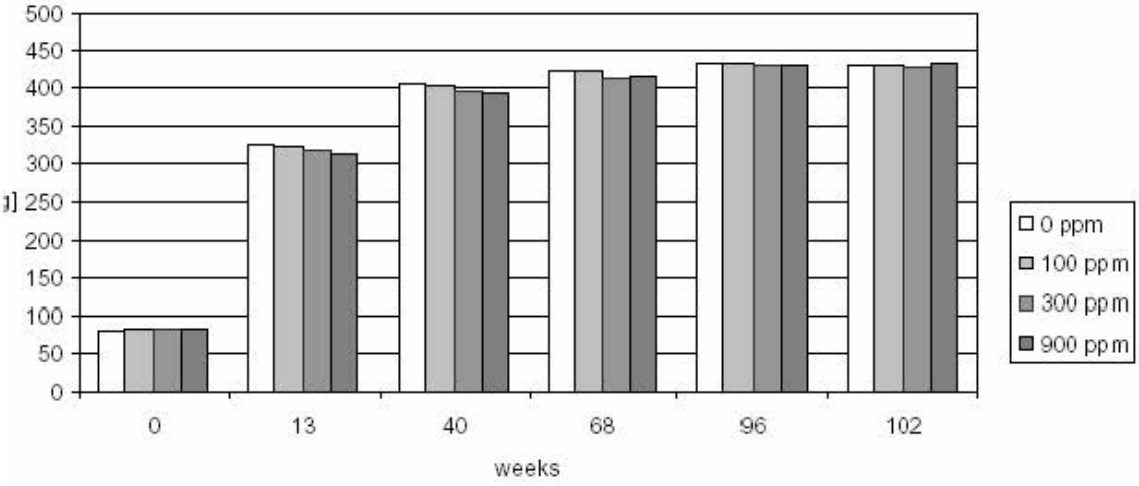

Figure A6.5/01 & /02-2: Supplement chronic study in rats - Body weights (mean values) males

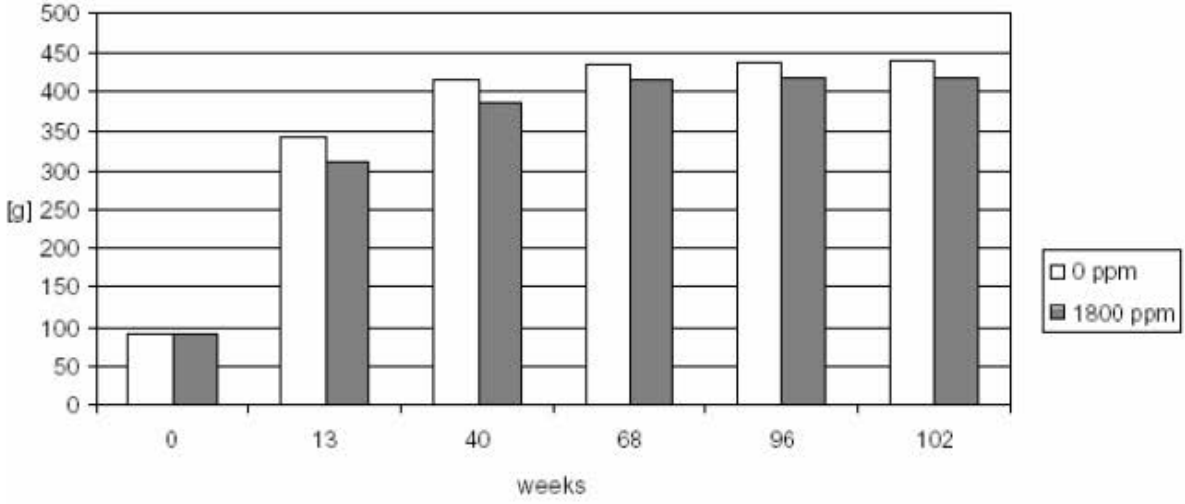

Figure A6.5/01 & /02-3: Main chronic study in rats - Body weights (mean values) females

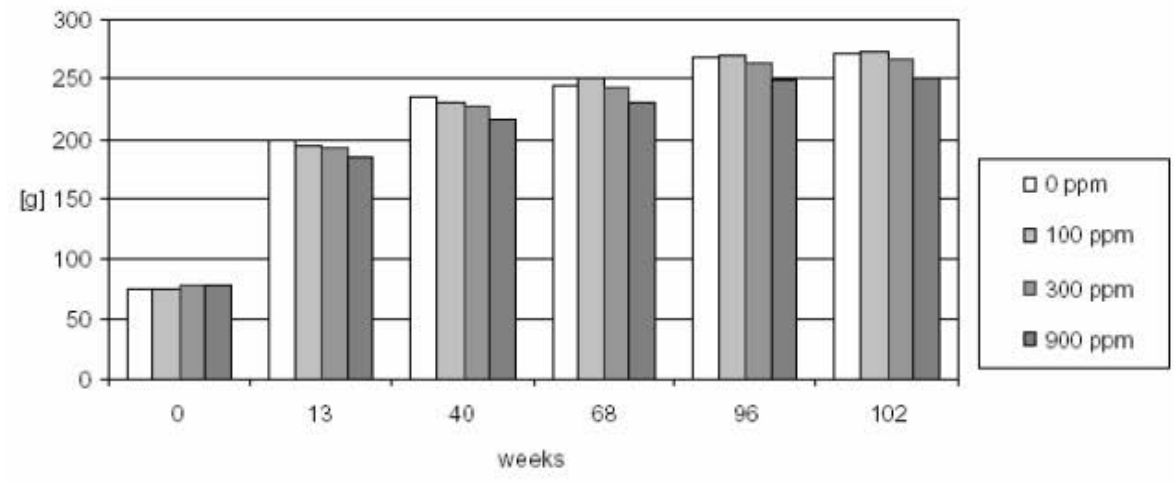

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Figure A6.5/01 & /02-4: Supplement chronic study in rats - Body weights (mean values) females

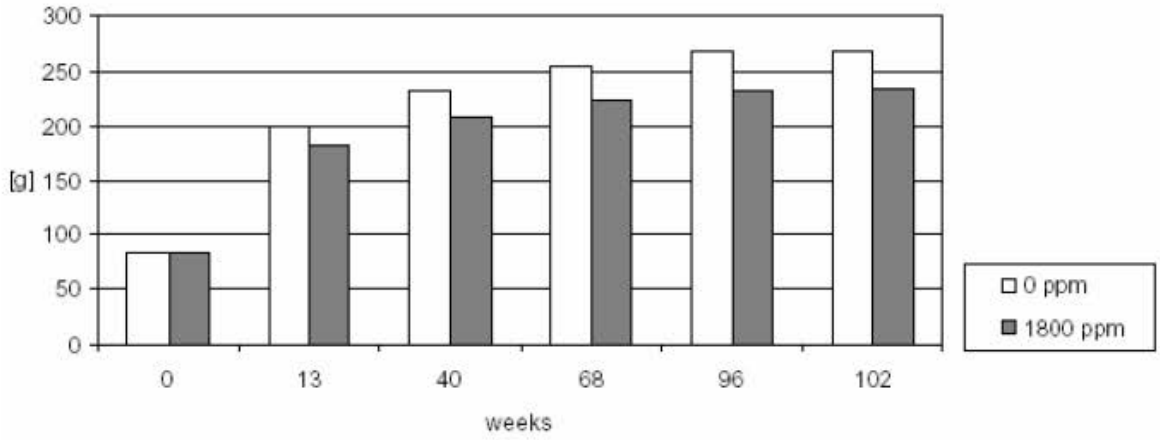

Table A6.5/01 & /02-1. Chronic study in rats-Absolute organ weights after 12 months

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

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

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Table A6.5/01 & /02-2. Chronic study in rats-Histopathological findings

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

CA-Table 1 – Body weights – week 102

	male		female	
0 ppm	432	(100 %)	272	(100 %)
100 ppm	430	(100 %)	273	(100 %)
300 ppm	428	(99 %)	266	(98 %)
900 ppm	434	(100 %)	251	(92 %)
0 ppm	433	(100 %)	269	(100 %)
1800 ppm	414	(96 %)	**236**	**(88 %)**

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CA-Table 2 – Absolute and relative organ weights - females at terminal necropsy

	BW (g)	Heart	Liver	Spleen	Kidney	Adrenals
Absolute organ weights (mg)						
0 ppm	269	1302	10121	551	1971	78
1800 ppm	236*	1101**	8414**	476**	1746**	65**
Relative organ weights (mg)						
0 ppm	269	488	3790	206	684	30
1800 ppm	236*	468	3578*	202	673	28

* $p \leq 0.05$, ** $p \leq 0.01$ (Man-Whitney + Wilcoxon test, two-sided)

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Section A6.5/03 **Chronic toxicity**

Section A6.5/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.5

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1 REFERENCE

1.1	**Reference**	*PPP Monograph B6.5.2, II A, 5.5.2 /01and 02*
	Authors (year)	[redacted] (1991 a and b)
	Title	a) NTN 33893 (proposed common name Imidacloprid) - Carcinogenicity study on B6C3F1 mice (administration in the food for 24 months) b) NTN 33893 (proposed common name: Imidacloprid) - Carcinogenicity study in B6C3F1 mice (supplementary MTD testing for study T5025710 with administration in diet over a 24-month period)
	Company, report No.	Bayer CropScience AG, Report-No.: a) 19931 b) 20769 BES Ref. : a) M-026310-01-1 b) M-026038-01-1
	Date	a) 1991-01-28 b) 1991-10-24
	Testing facility	[redacted]
	Dates of work	a) September 1987 – September 1989 b) September 1988 – September 1990
	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		
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 compliance with OECD 451, FIFRA, § 83-2.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, mixed batch no.180587, purity: 95.3 %; supplementary study: 95.0 %
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	

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Section A6.5/03 Chronic toxicity

Section A6.5/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.5

3.2	**Test Animals**	
3.2.1	Species	Male and female B6C3F1 mice (Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	18-25 g males / 14-18 g females / 5 weeks old
3.2.6	Number of animals per group	50 m, 50f/dose group in main and supplemental study 10m, 10f/dose group as 12 month sacrifice in both studies
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	24 months
3.3.2	Frequency of exposure	Continual in diet
3.3.3	Postexposure period	All animals sacrificed at the end of the exposure period
3.3.4	**Oral**	
3.3.4.1	Type	Main study in the diet at concentrations of 0, 100, 330 and 1000 ppm
3.3.4.2	Concentration	In a supplement MTD study, groups of 50 male and female mice were administered imidacloprid at levels of 0 and 2000 ppm in their diet for 24 months. Mean consumption of imidacloprid per kg body weight per day was 20.2, 65.6, 208.2, or 413.5 mg for males and 30.3, 103.6, 274.4, or 423.9 mg for females.
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	Yes, per OECD 451, FIFRA, § 83-2, no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.4.1.1	Clinical signs	
3.4.1.2	Mortality	
3.4.2	Body weight	
3.4.3	Food consumption	
3.4.4	Water consumption	

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Section A6.5/03 **Chronic toxicity**

Section A6.5/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.5

3.4.5	Ophthalmoscopic examination		
3.4.6	Haematology		
3.4.7	Clinical Chemisty		
3.4.8	Urinalysis		
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, per OECD 451, FIFRA, § 83-2, no deviations noted by the RMS in the December 2005 91/414 draft DAR	
3.5.2	Gross and histopathology		
3.5.3	Other examinations		
3.5.4	Statistics	Mann-Whitney & Wilcoxon 2-tailed U-test, generalized Wilcoxin test, generalized Kuskal-Wallis test	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Unusual vocalisations, squeaking and twittering, which increased whenever the animals became agitated were observed throughout the study in males and females at 2000 ppm. In addition, hypersensitivity to ether narcosis and/or blood withdrawal resulting in increased mortality after manipulations was observed in this dose group. Nine males and four females died after anaesthesia for tattooing or blood sampling compared with no mortalities in the control group. Similar observations were made in the 15-week range-finding study (non-key study).	X
4.1.2	Mortality		
4.2	**Body weight gain**	See Table A6.5/03 & /04-1	
4.3	**Food consumption and compound intake**	Food intake was slightly reduced in females at 1000 ppm and markedly reduced in females (-24 %) at 2000 ppm. Water intake was decreased slightly in females at 1000 ppm and markedly in males (-11 %) and females (-27 %) at 2000 ppm. See Table A6.5/03 & /04-2.	
		Body weight development was not influenced at doses up to and including 330 ppm. At 1000 ppm the mice exhibited reduced weight gain and marked reductions were seen at 2000 ppm (up to -29 % in males and -26 % in females).	X
4.4	**Ophtalmoscopic examination**	No treatment related effects noted.	
4.5	**Blood analysis**		
4.5.1	Haematology	No indications of treatment-related haemotoxicity or damage to the haematogenic organs was found at 1000 ppm. At 2000 ppm lower leukocyte counts were determined in both sexes. The clinical chemistry gave no evidence for liver damage. Reduced blood cholesterol levels at 2000 ppm indicate an effect on the lipid metabolism in this group. Kidney related clinical chemistry parameters in the blood were unaffected in the 2000 ppm group. See Table A6.5/03 & /04-3.	X
4.5.2	Clinical chemistry		
4.5.3	Urinalysis		

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Section A6.5/03 **Chronic toxicity**

Section A6.5/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.5

4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Morphological evidence for a marginal functional effect on the liver (low-grade periacinal hepatic cell hypertrophy) was found in a few males at 2000 ppm. This marginal change probably results from hepatocyte adaptation to the foreign substance metabolism, and should not be interpreted as evidence for liver damage. At 2000 ppm more animals than in the control groups exhibited mineralisation of the thalamic region of the brain. The region of brain mineralisation is only identified in the report on the MTD study but not in the main study, so that a direct comparison with the findings of the first study is not possible (see Table A6.5/03 & /04-4).	X
4.6.2	Gross and histopathology		
4.7	**Other**	The nature, location, incidence and latency periods of the detected tumors presented no evidence for an oncogenic effect.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In an oncogenicity study done in complance with OECD 451, FIFRA, § 83-2 guidelines, imidacloprid was administered to groups of 50 male and 50 female mice in their diet at concentrations of 0, 100, 330 and 1000 ppm for 24 months. In a supplement MTD study, groups of 50 male and female mice were administered imidacloprid at levels of 0 and 2000 ppm in their diet for 24 months. Ten additional mice per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was 20.2, 65.6, 208.2, or 413.5 mg for males and 30.3, 103.6, 274.4, or 423.9 mg for females.	
5.2	**Results and discussion**	Unusual vocalisations, squeaking and twittering, which increased whenever the animals became agitated, were observed throughout the study in males and females at 2000 ppm. In addition, hypersensitivity to ether narcosis and/or blood withdrawal resulting in increased mortality after manipulations was observed in this dose group. Nine males and four females died after anaesthesia for tattooing or blood sampling compared with no mortalities in the control group.	X
		Food intake was slightly reduced in females at 1000 ppm and markedly reduced in females (-24 %) at 2000 ppm. Water intake was decreased slightly in females at 1000 ppm and markedly in males (-11 %) and females (-27 %) at 2000 ppm. Body weight development was not influenced at doses up to and including 330 ppm. At 1000 ppm the mice exhibited reduced weight gain and marked reductions were seen at 2000 ppm (up to -29 % in males and -26 % in females).	X
		No indications of treatment-related haemotoxicity or damage to the haematogenic organs was found at 1000 ppm. At 2000 ppm lower leukocyte counts were determined in both sexes. The clinical chemistry gave no evidence for liver damage. Reduced blood cholesterol levels at 2000 ppm indicate an effect on the lipid metabolism in this group. Kidney related clinical chemistry parameters in the blood were unaffected in the 2000 ppm group.	X

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Section A6.5/03 **Chronic toxicity**

Section A6.5/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.5

		Morphological evidence for a marginal functional effect on the liver (low-grade periacinal hepatic cell hypertrophy) was found in a few males at 2000 ppm. This marginal change probably results from hepatocyte adaptation to the foreign substance metabolism, and should not be interpreted as evidence for liver damage. At 2000 ppm more animals than in the control groups exhibited mineralisation of the thalamic region of the brain. The region of brain mineralisation is only identified in the report on the MTD study but not in the main study, so that a direct comparison with the findings of the first study is not possible	
5.3	**Conclusion**		
5.3.1	LO(A)EL	1000 ppm in males and females, respectively , based on reduced body weights	X
5.3.2	NO(A)EL	330 ppm (males/females), equivalent to 65.5 mg/kg bw/day for males and 103.6 mg/kg bw/day for females	X
5.3.3	Other	The nature, location, incidence and latency periods of the detected tumors presented no evidence for an oncogenic effect.	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/06
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.1.2/5.2 Nine males and four females in the treated groups died due to an increased sensitivity to ether anaesthesia for tattooing or blood sampling compared with one mortality in the control group. Despite the fact that marking animals by tattooing only 6 wk after study begin appears unusual, it is noted that this indirectly substance-related mortality was significantly increased in high-dose males whereas for females, no significance was obtained. However, this is obviously due to an unusually high early mortality rate in the female control group (4 dead animals by study wk 12), the reasons for which were not clearly explained in the study report. 4.2/5.2 Body weight development was not markedly (< 10 %) influenced at doses up to and including 1000 ppm. At 2000 ppm, body weight gain was decreased up to 25 % in males and up to 20 % in females (see CA-Table 1). 4.5.2/5.2 Significant elevation of AP in the 2000 ppm group is indicative for liver toxicity (see CA-Table 2). 4.6.1/5.2 Absolute and relative liver weights were significantly decreased in females at 2000 ppm. At 2000 ppm, more animals than in the control groups exhibited mineralisation of the thalamic region of the brain, however, the number of animals affected did not differ significantly from the control group.
Conclusion	Different from the applicant's version, the LOAEL and NOAEL are as follows: LOAEL: 414/424 mg/kg bw/d (M/F, 2000 ppm) based on markedly (> 10 %) decreased body weight, liver effects (hepatocyte hypertrophy, decreased liver weight and elevated AP) and decreased cholesterol NOAEL: 208/274 mg/kg bw/d (M/F, 1000 ppm)
Reliability	2
Acceptability	Acceptable with restrictions; since this study is a carcinogenicity study in accordance with OECD 451 and not a combined chronic toxicity/carcinogenicity study, there are some deviations from the requirements for a chronic study (as laid down in OECD 453): satellite groups consisted of 10 instead of 20 animals, no urinalysis was performed, blood examinations were carried out every 12 instead of every 6 months.
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*

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Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.5/03 & /04-1. Chronic study in mice-Mean body weights

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

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

Table A6.5/03 & /04-2. Supplement chronic study in mice-Food and water intake

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

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Table A6.5/03 & /04-3. Chronic study in mice-Haematology and clinical chemistry

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

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

Table A6.5/03 & /04-4. Chronic study in mice-Histopathological findings

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

CA-Table 1: Mean body weight (% of control)

	100 ppm	330 ppm	1000 ppm	2000 ppm
Males				
Week 0	98,1	96,1	98,1	104,2
Week 13	98,9	97,5	96,0	**87,1**
Week 27	99,7	96,7	95,1	**79,4**
Week 41	97,2	97,8	93,4	**81,1**
Week 55	97,9	96,0	94,5	**75,0**
Week 69	99,7	97,6	94,3	**75,0**
Week 83	99,1	99,1	95,9	**75,0**
Week 97	96,8	98,8	95,4	**76,9**
Week 104	97,9	100,0	97,3	**75,0**
Females				
Week 0	96,9	95,7	98,2	100,0
Week 13	98,8	98,0	100,4	**88,9**
Week 27	98,1	97,7	98,9	**86,2**
Week 41	100,0	99,3	98,9	**86,7**
Week 55	99,3	98,6	100,4	**81,3**
Week 69	98,0	96,9	98,6	**79,4**
Week 83	100,0	97,6	99,0	**79,4**
Week 97	99,0	98,6	98,3	**84,4**
Week 104	99,7	98,3	99,3	**79,4**

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CA-Table 2: Clinical Chemistry – 0 ppm and 2000 ppm group

KLINISCHE CHEMIE (BLUT) / CLINICAL CHEMISTRY (BLOOD)

Dosis/ dose (ppm)	Woche/ week	ASAT (GOT) U/l	ALAT (GPT) U/l	APh U/l	GLUCOSE mmol/l	CHOL mmol/l	CREA mcmol/l	HST UREA mmol/l	BILI-t mcmol/l	PROT g/l
MAENNLICH / MALE										
0	54	20.7	27.4	93	6.68	3.54	28	12.23	2.0	57.9
2000	54	29.8**	35.9	123**	5.85**	2.55**	28	10.96	2.1	54.9**
0	104	26.9	34.0	116	5.92	4.29	25	12.35	1.8	60.5
2000	104	34.1	27.6	157**	6.03	2.85**	24	9.68**	1.9	56.5
WEIBLICH / FEMALE										
0	54	30.2	31.0	168	6.06	2.40	28	10.96	2.5	56.8
2000	54	34.1	36.5	249**	5.90	2.13*	29	10.74	3.2**	54.3
0	104	56.3	66.3	434	5.71	2.58	24	10.40	3.0	55.9
2000	104	33.4	32.0	661	5.98	2.34	27*	9.46	3.5	56.1

* $p \leq 0.05$, ** $p \leq 0.05$ (Mann-Whitney U-test, two-tailed)

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Section A6.6.1/01

Annex Point IIA6.6.1/01

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /01*	
	Authors (year)	[redacted] (1989a)	
	Title	NTN 33893 - Salmonella/microsome test to evaluate for point mutagenic effects	
	Company, report No.	Bayer CropScience AG, Report-No.: 17577 BES Ref. : M-027611-01-1	
	Date	1989-01-06	
	Testing facility	[redacted]	
	Dates of work	November 1988	
	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			
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 471, 84/449/EEC, FIFRA-PB 84-233295	X
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587 purity: 95.0 %	
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	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98	
3.2.2	Metabolic activation system	S9 mix	
3.2.3	Positive control	Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene	

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Section A6.6.1/01

Annex Point IIA6.6.1/01

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested in the Salmonella/microsome assay at concentrations of up to and including 12500 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Per 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**	Imidacloprid concentrations up to 6200 µg/plate did not produce an increase in the mutant count. The total bacteria counts remained unchanged. No inhibition of growth was observed. At higher doses, the substance had a very weak strain-specific bacteriotoxic effect ; this range could not be used for evaluation purposes.
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 OECD 471, 84/449/EEC, FIFRA-PB 84-233295, imidacloprid was tested in the Salmonella/microsome assay at concentrations of up to and including 12500 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.
5.2	**Results and discussion**	Imidacloprid concentrations up to 6200 µg/plate did not produce an increase in the mutant count. The total bacteria counts remained unchanged. No inhibition of growth was observed. At higher doses, the substance had a very weak strain-specific bacteriotoxic effect; this range could not be used for evaluation purposes.
5.3	**Conclusion**	Imidacloprid is considered to be non-mutagenic in this assay with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Section A6.6.1/01

Annex Point IIA6.6.1/01

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Imidacloprid is considered to be non-mutagenic in this assay in the tested strains with and without metabolic activation.
Reliability	1
Acceptability	Acceptable
Remarks	2.1 Study in accordance with OECD 471 (26th May 1983) 2.3 Deviations from OECD 471 (21st July 1997): strain for detection of cross-linking agents (TA102 or E.coli WP2) missing; 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	

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Section A6.6.1/02

Annex Point IIA6.6.1

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /02*	
	Authors (year)	[redacted] (1991)	
	Title	NTN 33893 AMP - Salmonella/microsome test	
	Company, report No.	Bayer CropScience AG, Report-No.: 20090 BES Ref. : M-025825-01-1	
	Date	1991-03-22	
	Testing facility	[redacted]	
	Dates of work	January 1991	
	Test substance(s)	Molecule(s): imidacloprid Substance(s): NTN 33893 AMP Z (Batch-No.: 17133/90)	
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 471, 84/449/EEC, FIFRA-PB 84-233295	X
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid AMP, batch no. 17133/90 purity: 96.0-96.3 %
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	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene

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Section A6.6.1/02

Annex Point IIA6.6.1

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid AMP was tested in the Salmonella/microsome assay at concentrations of up to and including 5000 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Per 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**	Imidacloprid AMP concentrations of up to 5000 µg/plate did not cause any mutagenic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed.
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 OECD 471, 84/449/EEC, FIFRA-PB 84-233295, imidacloprid AMP was tested in the Salmonella/microsome assay at concentrations of up to and including 5000 µg/plate. The solvent was DMSO. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537 and TA 98. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls. The tests were done with and without addition of S9 mix.
5.2	**Results and discussion**	Imidacloprid AMP concentrations of up to 5000 µg/plate did not cause any mutagenic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed.
5.3	**Conclusion**	Imidacloprid is considered to be non-mutagenic in this assay with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Section A6.6.1/02

Annex Point IIA6.6.1

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Imidacloprid is considered to be non-mutagenic in this assay in the tested strains with and without metabolic activation.
Reliability	1
Acceptability	Acceptable
Remarks	2.1 Study in accordance with OECD 471 (26th May 1983) 2.3 Deviations from OECD 471 (21st July 1997): strain for detection of cross-linking agents (TA102 or E.coli WP2) missing; 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	

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Section A6.6.1/03

Annex Point IIA6.6.1/03

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /03*	
	Location in dossier	II A, 5.4.1 /03	
	Authors (year)	[redacted] (1992)	
	Title	NTN 33893 AMP W - Salmonella/microsome test	
	Company, report No.	Bayer CropScience AG, Report-No.: 21775 BES Ref. : M-029085-01-1	
	Date	1992-10-19	
	Testing facility	[redacted]	
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR (Batch-No.: 816 255 007)	
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 471, 84/449/EEC, FIFRA-PB 84-233295	X
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid AMP W, CONFIDOR (Batch-No.: 816 255 007) purity: 97.4 %	
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	**Study Type**	Bacterial reverse mutation test	
3.2.1	Organism/cell type	S. typhimurium: TA 1535, TA 100, TA 1537, TA 98	
3.2.2	Metabolic activation system	S9 mix	
3.2.3	Positive control	Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene	

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Section A6.6.1/03

Annex Point IIA6.6.1/03

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested in the Salmonella/microsome test at doses up and including 5000 µg/plate in the bacteria strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activations.Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene 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**	
3.4.1	Number of cells evaluated	Per 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**	There was no evidence for mutagenic effects of imidacloprid AMP W 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 OECD 471, 84/449/EEC, FIFRA-PB 84-233295, imidacloprid AMP was tested in the Salmonella/microsome test at doses up and including 5000 µg/plate in the bacteria strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activations. Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive controls.
5.2	**Results and discussion**	There was no evidence for mutagenic effects of imidacloprid AMP W with and without metabolic activation.
5.3	**Conclusion**	Imidacloprid AMP W is considered to be negative in the Salmonella/microsome test with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Imidacloprid AMP W is considered to be non-mutagenic in this assay in the tested strains with and without metabolic activation.
Reliability	1
Acceptability	Acceptable
Remarks	2.1 Study in accordance with OECD 471 (26th May 1983) 2.3 Deviations from OECD 471 (21st July 1997): strain for detection of cross-linking agents (TA102 or E.coli WP2) missing; 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	

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Section A6.6.1/04

Annex Point IIA6.6.1

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium and Escherichia coli

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /04*	
	Authors (year)	[redacted] 1991a)	
	Title	NTN 33893 - Reverse mutation assay (Salmonella typhimurium and Escherichia coli)	
	Company, report No.	Bayer CropScience AG, Report-No.: RA91002 BES Ref. : M-028670-01-1	
	Date	1991-01-17	
	Testing facility	[redacted]	
	Dates of work	October 1990	
	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			
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 471, 84/449/EEC, FIFRA-PB 84-233295	X
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid Batch-No.: 180587 purity: 93.7 %	
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	**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	2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide, 2-Aminoanthracene, N-Ethyl-N'-nitro-N-nitrosoguanidine and 9-Aminoacridine	

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Section A6.6.1/04

Annex Point IIA6.6.1

Genotoxicity in vitro / In-vitro gene mutation study in bacteria

Gene mutation in Salmonella typhimurium and Escherichia coli

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for mutagenic effects with and without metabolic activation using Salmonella typhimurium TA98, TA100, TA1535 and TA1537 strains as well as Escherichia coli WP2/uvrA strain up to and including 5000 µg per plate. The solvent was DMSO. 2-(2-Furyl)-3-(5-nitro-2- furyl) acrylamide, 2-Aminoanthracene, N-Ethyl-N'-nitro-N-nitrosoguanidine and 9-Aminoacridine were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Per 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**	Imidacloprid concentrations of up to and including 5000 µg/plate did not produce an increase in the mutant frequency. No bacteriotoxic effect occurred. The positive controls demonstrated a good sensitivity of this assay.
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 OECD 471, 84/449/EEC, FIFRA-PB 84-233295, imidacloprid was tested for mutagenic effects with and without metabolic activation using Salmonella typhimurium TA98, TA100, TA1535 and TA1537 strains as well as Escherichia coli WP2/uvrA strain up to and including 5000 µg per plate. The solvent was DMSO. 2-(2-Furyl)-3-(5-nitro-2- furyl) acrylamide, 2-Aminoanthracene, N-Ethyl-N'-nitro-N-nitrosoguanidine and 9-Aminoacridine were used as positive controls.
5.2	**Results and discussion**	Imidacloprid concentrations of up to and including 5000 µg/plate did not produce an increase in the mutant frequency. No bacteriotoxic effect occurred. The positive controls demonstrated a good sensitivity of this assay.
5.3	**Conclusion**	Imidacloprid is considered to be non-mutagenic in these assays with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
Materials and Methods	Applicant' version is acceptable.
Results and discussion	Applicant' version is acceptable.
Conclusion	Imidacloprid is considered to be non-mutagenic in this assay with and without metabolic activation.
Reliability	1
Acceptability	Acceptable
Remarks	2.1 Study in accordance with OECD 471 (26th May 1983) 2.3. Deviations from OECD 471 (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	

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Section A6.6.2/01

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity in Chinese Hamster Ovary Cells

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /09*
	Authors (year)	[redacted] (1988)
	Title	Clastogenic evaluation of NTN 33893 in an in vitro cytogenetic assay measuring sister chromatid exchange in chinese hamster ovary (CHO) cells
	Company, report No.	Bayer CropScience AG, Report-No.: R4407 BES Ref. : M-026488-01-1
	Date	1988-04-21
	Testing facility	[redacted]
	Dates of work	February 1988
	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		
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, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.2 %
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	**Study Type**	*In vitro* sister chromatid exchange assay in mammalian cells
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	Mitomycin-C for the non-activation and cyclophosphamide in the metabolic activation series

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Section A6.6.2/01

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity in Chinese Hamster Ovary Cells

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for clastogenic activity in the *in vitro* cytogenetic assay measuring sister chromatid exchange in Chinese hamster ovary cells without and with S9-mix up to and including 5000µg/mL. The solvent was DMSO. Mitomycin-C for the non-activation and cyclophosphamide in the metabolic activation series were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.3.5	Number of cells evaluated	Per OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986, 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	A statistically significant increase in SCE-rate at concentrations of 250 – 1000 µg/mL (without S9-mix) and of 2000 – 3000 µg/mL (with S9-mix). Mitotic inhibition and cell cycle delay were observed in the same concentration range.
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, Imidacloprid was tested for clastogenic activity in the *in vitro* cytogenetic assay measuring sister chromatid exchange in Chinese hamster ovary cells without and with S9-mix up to and including 5000µg/mL. The solvent was DMSO. Mitomycin-C for the non-activation and cyclophosphamide in the metabolic activation series were used as positive controls
5.2	**Results and discussion**	A statistically significant increase in SCE-rate at concentrations of 250 – 1000 µg/mL (without S9-mix) and of 2000 – 3000 µg/mL (with S9-mix). Mitotic inhibition and cell cycle delay were observed in the same concentration range.
5.3	**Conclusion**	Imidacloprid was found to induce weak clastogenic effects in CHO cells *in vitro*.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Section A6.6.2/01

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity in Chinese Hamster Ovary Cells

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.2/02

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity in Chinese Hamster Ovary Cells

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /10*
	Authors (year)	[redacted] (1989)
	Title	BAY NTN 33893 - Sister chromatid exchange assay in chinese hamster ovary cells
	Company, report No.	Bayer CropScience AG, Report-No.: BC1149 BES Ref. : M-025499-01-1
	Date	1989-09-12
	Testing facility	[redacted]
	Dates of work	October 1988
	Test substance(s)	Molecule(s): imidacloprid Substance(s): imidacloprid techn. Batch No.: 17001/88
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, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. PF 17001/88, purity: 95.2 %
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	**Study Type**	*In vitro* sister chromatid exchange assay in mammalian cells
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	Triethylenemelamine and cyclophosphamide

Property of Bayer Environmental Science

Section A6.6.2/02

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity in Chinese Hamster Ovary Cells

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for clastogenic effects in the sister chromatic assay both in the absence and presence of S9-mix activation system at dose levels of 25, 50, 100, 200 and 400 µg/mL in nonactivated study and 157, 313, 625 and 1250 µg/mL in the S9-mix activated study. The solvent was DMSO. Triethylenemelamine and cyclophosphamide were used as the positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.3.5	Number of cells evaluated	Per OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986, 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	Imidacloprid did not induce a relevant increase in sister chromatid exchange without and with S9- mix. The positive controls demonstrated a good sensitivity of this assay.
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, imidacloprid was tested for clastogenic effects in the sister chromatic assay both in the absence and presence of S9-mix activation system at dose levels of 25, 50, 100, 200 and 400 µg/mL in nonactivated study and 157, 313, 625 and 1250 µg/mL in the S9-mix activated study. The solvent was DMSO. Triethylenemelamine and cyclophosphamide were used as the positive controls.
5.2	**Results and discussion**	Imidacloprid did not induce a relevant increase in sister chromatid exchange without and with S9- mix. The positive controls demonstrated a good sensitivity of this assay.
5.3	**Conclusion**	Imidacloprid is considered not to induce clastogenic effects in CHO cells in this test.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.2/03

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity with Human Lymphocytes

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /11*
	Authors (year)	[redacted] (1989b)
	Title	NTN 33893 - In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects
	Company, report No.	Bayer CropScience AG, Report-No.: 18092 BES Ref. : M-028377-02-1
	Date	1989-06-16, Amended 1989-08-24
	Testing facility	[redacted]
	Dates of work	May 1988
	Test substance(s)	Molecule(s): imidacloprid Substance(s): NTN 33893 Z (Batch No.: 880226ELB01, 180587)
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, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, main study: batch no. 180587, purity: 95.2 %; addendum: batch no. 880226ELB01, 99.8 %
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	**Study Type**	*In vitro* mammalian cell gene mutation test
3.2.1	Organism/cell type	human lymphoblastoid cells
3.2.2	Metabolic activation system	S9 mix
3.2.3	Positive control	Mitomycin C and cyclophoshamide

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Section A6.6.2/03

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity with Human Lymphocytes

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was evaluated in the human lymphocyte cytogenetics assay *in vitro* for clastogenic effects at concentrations of up to 5200 µg/mL without and with S9-mix. The solvent was DMSO. Mitomycin C and cyclophoshamide were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.3.5	Number of cells evaluated	Per OECD 473, 92/69/EEC B.10., US-EPA-FIFRA Subpart F-Genetic Toxicology, Rev. July 1, 1986, 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	Imidacloprid showed a clastogenic effect without S9-mix at cytotoxic concentrations of 500 – 5200 µg/mL. With S9-mix a weak clastogenic effect could not be ruled out. 50 µg/mL were without any effects. The positive controls had a clear clastogenic effect. In the second study the clastogenic effect of imidacloprid was reproduced . This supports the conclusion that the clastogenic effect was not due to by-products present in the batch used for the first study.
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, imidacloprid was evaluated in the human lymphocyte cytogenetics assay *in vitro* for clastogenic effects at concentrations of up to 5200 µg/mL without and with S9-mix. The solvent was DMSO. Mitomycin C and cyclophoshamide were used as positive controls.
5.2	**Results and discussion**	Imidacloprid showed a clastogenic effect without S9-mix at cytotoxic concentrations of 500 – 5200 µg/mL. With S9-mix a weak clastogenic effect could not be ruled out. 50 µg/mL were without any effects. The positive controls had a clear clastogenic effect. In the second study the clastogenic effect of imidacloprid was reproduced . This supports the conclusion that the clastogenic effect was not due to by-products present in the batch used for the first study.
5.3	**Conclusion**	Imidacloprid is considered to induce clastogenic effects in human lymphocytes *in vitro*.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Section A6.6.2/03

Annex Point IIA6.6.2

Genotoxicity in vitro / In-vitro cytogenicity study in mammalian cells

Cytogenicity with Human Lymphocytes

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
Materials and Methods	Applicant' version is acceptable.
Results and discussion	Applicant' version is acceptable.
Conclusion	Applicant' 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	

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Section A6.6.3/01

Annex Point IIA6. 6.3

Genotoxicity in vitro / In-vitro gene mutation assay in mammalian cells

Forward mutation in the CHO-HGPRT assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /06*
	Authors (year)	[redacted] (1989a)
	Title	NTN 33893 - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay in vitro
	Company, report No.	Bayer CropScience AG, Report-No.: 17578 BES Ref. : M-027630-01-1
	Date	1989-01-06
	Testing facility	[redacted]
	Dates of work	April – July 1988
	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		
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**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.2 %
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	**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.

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Section A6.6.3/01

Annex Point IIA6. 6.3

Genotoxicity in vitro / In-vitro gene mutation assay in mammalian cells

Forward mutation in the CHO-HGPRT assay

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for mutagenic effects at the HGPRT locus (forward mutation assay) in CHO cell cultures (CHO-K1-BH4) after *in vitro* treatment at concentrations up to 125 µg/mL without S-9 mix and 1222 µg/mL with S-9 mix. The solvent was DMSO. Ethanemethanesulfonate (EMS) was used as a positive control without S9 mix, and dimethylbenzanthracene (DMBA) as a positive control with S9 mix.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	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	Imidacloprid concentrations of up to 125 µg/mL without metabolising enzyme fraction and 1222 µg/mL with S-9 mix did not produce an increase in the mutant frequency. Cytotoxic effects with a relative survival < 50 % were observed at ≥ 90 µg/mL without and at ≥ 800 µg/mL with S-9 mix. The positive controls demonstrated a good sensitivity of this assay.
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, imidacloprid technical was tested for mutagenic effects at the HGPRT locus (forward mutation assay) in CHO cell cultures (CHO-K1-BH4) after treatment at concentrations up to 125 µg/mL without S-9 mix and 1222 µg/mL with S-9 mix. The solvent was DMSO. Ethanemethanesulfonate (EMS) was used as a positive control without S9 mix, and dimethylbenzanthracene (DMBA) as a positive control with S9 mix
5.2	**Results and discussion**	Imidacloprid concentrations of up to 125 µg/mL without metabolising enzyme fraction and 1222 µg/mL with S-9 mix did not produce an increase in the mutant frequency. Cytotoxic effects with a relative survival < 50 % were observed at ≥ 90 µg/mL without and at ≥ 800 µg/mL with S-9 mix. The positive controls demonstrated a good sensitivity of this assay.
5.3	**Conclusion**	Imidacloprid is considered to be non-mutagenic in the HGPRT test with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Section A6.6.3/01

Annex Point IIA6. 6.3

Genotoxicity in vitro / In-vitro gene mutation assay in mammalian cells

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	2007/02/08
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	

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Section A6.6.3/02 Genotoxicity in vitro / In-vitro gene mutation assay

Annex Point IIA6. 6.3

Mitotic recombination assay with saccharomyces cerevisiae

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /07*
	Authors (year)	[redacted] (1988a)
	Title	NTN 33893 - Test on S. cerevisiae D7 to evaluate for induction of mitotic recombination
	Company, report No.	Bayer CropScience AG, Report-No.: 16832 BES Ref. : M-027595-01-1
	Date	1988-06-27
	Testing facility	[redacted]
	Dates of work	September – October 1987
	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		
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 480
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.3 %
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	**Study Type**	Mitotic recombination assay with saccharomyces cerevisiae
3.2.1	Organism/cell type	yeast: saccharomyces cerevisiae
3.2.2	Positive control	Methyl methane sulphonate and cyclophosphamide

Property of Bayer Environmental Science

Section A6.6.3/02 Genotoxicity in vitro / In-vitro gene mutation assay

Annex Point IIA6. 6.3

Mitotic recombination assay with saccharomyces cerevisiae

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for induction of mitotic recombination (gene conversion and crossing-over) using Saccharomyces cerevisiae, strain D7, at concentrations of up to 10000 µg/mL. The solvent was DMSO. Methyl methane sulphonate and cyclophosphamide were used as positive controls.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Per OECD 480, no deviations noted by the RMS in the December 2005 91/414 draft DAR

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	Imidacloprid concentrations of up to and including 10000 µg/mL did not produce an increase in the mutant frequency. Evidence for induction of mitotic recombination by imidacloprid was not found. The positive controls demonstrated a good sensitivity of this assay.
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 480 guideline, imidacloprid technical was tested for induction of mitotic recombination (gene conversion and crossing-over) using Saccharomyces cerevisiae, strain D7, at concentrations of up to 10000 µg/mL. The solvent was DMSO. Methyl methane sulphonate and cyclophosphamide were used as positive controls.
5.2	**Results and discussion**	Imidacloprid concentrations of up to and including 10000 µg/mL did not produce an increase in the mutant frequency. Evidence for induction of mitotic recombination by imidacloprid was not found. The positive controls demonstrated a good sensitivity of this assay.
5.3	**Conclusion**	Imidacloprid is considered to be non-mutagenic in this test system.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.3/03 Genotoxicity in vitro / Unscheduled DNA synthesis

Annex Point IIA6. 6.3

Rat primary hepatocyte unscheduled DNA synthesis assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.1, II A, 5.4.1 /08*
	Authors (year)	[redacted] (1988)
	Title	Mutagenicity test on NTN 33893 in the rat primary hepatocyte unscheduled DNA synthesis assay
	Company, report No.	Bayer CropScience AG, Report-No.: R4631 BES Ref. : M-026493-01-1
	Date	1988-12-21
	Testing facility	[redacted]
	Dates of work	February 1988
	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		
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, 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**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.2 %
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	**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

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Section A6.6.3/03 Genotoxicity in vitro / Unscheduled DNA synthesis

Annex Point IIA6. 6.3

Rat primary hepatocyte unscheduled DNA synthesis assay

3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	Imidacloprid was tested for induction of 3H-thymidine incorporation in the *in vitro* rat primary hepatocyte unscheduled DNA (UDS) assay. Rat primary hepatocytes were exposed to imidacloprid concentrations from about 750 µg/mL to 5.00 µg/mL. The solvent was DMSO. 2-Acetyl aminofluorene was used as the positive control.
3.3.2	Way of application	
3.3.3	Pre-incubation time	
3.3.4	Other modifications	
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Per OECD 482, FIFRA PB 84-233295, 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	Imidacloprid did not induce significant changes of nuclear labelling of rat primary hepatocytes in the concentration range tested. The positive control substance demonstrated a good sensitivity of this assay.
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 PB 84-233295, 88/302/EEC guidelines, imidacloprid technical was tested for induction of 3H-thymidine incorporation in the rat primary hepatocyte unscheduled DNA (UDS) assay. Rat primary hepatocytes were exposed to imidacloprid concentrations from about 750 µg/mL to 5.00 µg/mL. The solvent was DMSO. 2-Acetyl aminofluorene was used as the positive control.
5.2	**Results and discussion**	Imidacloprid did not induce significant changes of nuclear labelling of rat primary hepatocytes in the concentration range tested. The positive control substance demonstrated a good sensitivity of this assay.
5.3	**Conclusion**	Imidacloprid is considered not to be a DNA-damaging substance in the rat primary hepatocyte UDS assay.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4

Cytogenetic in-vivo-study of bone marrow in Chinese hamster

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.2, II A, 5.4.2 /01*
	Authors (year)	[redacted] (1989c)
	Title	NTN 33893 - In vivo cytogenetic study of the bone marrow in chinese hamster to evaluate for induced clastogenic effects
	Company, report No.	Bayer CropScience AG, Report-No.: 18557 BES Ref. : M-025903-01-1
	Date	1989-11-24
	Testing facility	[redacted]
	Dates of work	April – August 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		
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 475
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 94.6 %.
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	
3.1.2.3	Maximum tolerable dose	2000 mg/kg bw,
3.2	**Test Animals**	
3.2.1	Species	Chinese hamster *(Cricetulus griseus)*
3.2.2	Source	[redacted]
3.2.3	Sex	m and f
3.2.4	Age/weight at study initiation	8-12w/26-35 g

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Section A6.6.4/01 Genotoxicity in vivo

Annex Point IIA6.6.4

Cytogenetic in-vivo-study of bone marrow in Chinese hamster

3.2.5	Number of animals per group	5m + 5f per dose and sampling time
3.2.6	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Postexposure period	6, 24, 48 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 2000 mg as/kg bw
3.3.6	Total volume applied	10 ml/kg bw
3.3.7	Controls	Vehicle
3.3.8	Substance used as Positive Control	Cyclophophamide (monohydrate), 30 mg/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	bone marrow
		Number of animals: 10/time point
		Number of cells: Approximately 100 metaphases/experimental animal were examined for structural changes in the chromosomes
		Time points: 6, 24, 48 h after treatment or other
		Parameters: numbers and types of structural aberrations

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	The treated animals showed no symptoms. External appearance and physical activity were unaffected. However, four animals died due to the acute toxicity of 2000 mg/kg bw. Symptoms of intoxication were not reported for these animals.
4.2	**Genotoxicity**	No changes indicative of a clastogenic effect of imidacloprid were observed. The results for the positive control indicated a clear clastogenic effect.

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Section A6.6.4/01

Annex Point IIA6.6.4

Genotoxicity in vivo

Cytogenetic in-vivo-study of bone marrow in Chinese hamster

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an *in vivo* cytogenetic study conducted according to OECD 475 guideline, imidacloprid technical was tested for clastogenic effects using the cytogenetic test on bone marrow of Chinese hamster following a single oral treatment of 2000 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and dissolved in deionised water. Administration volume was 10 mL/kg bw. Metaphases were prepared at 6 hrs, 24 hrs and 48 hrs postdose.
5.2	**Results and discussion**	The treated animals showed no symptoms. External appearance and physical activity were unaffected. However, four animals died due to the acute toxicity of 2000 mg/kg bw. Symptoms of intoxication were not reported for these animals. Approximately 100 metaphases/experimental animal were examined for structural changes in the chromosomes No changes indicative of a clastogenic effect of imidacloprid were observed. The results for the positive control indicated a clear clastogenic effect.
5.3	**Conclusion**	Imidacloprid is found not to induce a clastogenic effect in Chinese hamster bone marrow *in vivo*.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.4/02 Genotoxicity in vivo

Annex Point IIA6.6.4 *Cytogenetic in-vivo-studysister chromatid exchange in bone marrow of Chinese hamster*

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.2, II A, 5.4.2 /03*
	Authors (year)	[redacted] (1989d)
	Title	NTN 33893 - Sister chromatid exchange in bone marrow of chinese hamsters *in vivo*
	Company, report No.	Bayer CropScience AG, Report-No.: 18093 BES Ref. : M-028379-02-1
	Date	1989-06-16, Amended: 1993-11-23
	Testing facility	[redacted]
	Dates of work	November 1988 – April 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
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 to OPPTS 870.5915.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.0 %.
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	
3.1.2.3	Maximum tolerable dose	2000 mg/kg bw,
3.2	**Test Animals**	
3.2.1	Species	Chinese hamster *(Cricetulus griseus)*
3.2.2	Source	[redacted]
3.2.3	Sex	m and f
3.2.4	Age/weight at study initiation	8-12w/28-32 g

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Section A6.6.4/02 Genotoxicity in vivo

Annex Point IIA6.6.4 *Cytogenetic in-vivo-studysister chromatid exchange in bone marrow of Chinese hamster*

3.2.5	Number of animals per group	5m + 5f per dose
3.2.6	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Postexposure period	24 h after treatment
		Oral
3.3.3	Type	gavage
3.3.4	Vehicle	0.5% aqueous Cremophor emulsion
3.3.5	Concentration in vehicle	Sufficient to deliver the equivalent to 500, 1000 or 2000 mg as/kg bw
3.3.6	Total volume applied	10 ml/kg bw
3.3.7	Controls	Vehicle
3.3.8	Substance used as Positive Control	Cyclophophamide (monohydrate), 10 mg/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	bone marrow
		Number of animals: 10/time point
		Number of cells: Approximately 500 bone marrow cells/experimental animal were examined and the ratio of mitotic to non-mitotic cells determined
		Time points: 24 h after treatment or other
		Parameters: Cytotoxicity and SCE-frequency

4 RESULTS AND DISCUSSION

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

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Section A6.6.4/02 Genotoxicity in vivo

Annex Point IIA6.6.4

Cytogenetic in-vivo-studysister chromatid exchange in bone marrow of Chinese hamster

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an *in vivo* cytogenetic study conducted in main accordance to OPPTS 870.5915 guideline, imidacloprid technical was tested for DNA-modifications using the sister chromatid exchange method following a single oral treatment of 500, 1000 and 2000 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control, dissolved in deionised water and applied at a single dose of 10 mg/kg bw. Administration volume was 10 mL/kg bw.
5.2	**Results and discussion**	The treated animals showed no symptoms. All animal survived until the end of the study. There was a slight but significant reduction (17 %) of the mitotic index occurred at 1000 mg/kg bw and 2000 mg/kg bw. No changes indicative of a clastogenic effect of imidacloprid were observed. The results for the positive control indicated a clear clastogenic effect.
5.3	**Conclusion**	Imidacloprid is found not to induce clastogenic effects (sister chromatid exchange) in bone marrow of Chinese hamster *in vivo*.
5.3.1	Reliability	1
5.3.2	Deficiencies	Only SCE per metaphase established

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/08
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	

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Section A6.6.5 Genotoxicity in vivo/ Second study

Annex Point IIA6.6.5

Mouse micronucleus study

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.2, II A, 5.4.2 /02*
	Authors (year)	[redacted] (1988b)
	Title	NTN 33893 - Micronucleus-test on the mouse to evaluate for clastogenic effects
	Company, report No.	Bayer CropScience AG, Report-No.: 16837 BES Ref. : M-027591-01-1
	Date	1988-06-27
	Testing facility	[redacted]
	Dates of work	September – November 1987
	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		
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**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 95.3 %
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	
3.1.2.3	Maximum tolerable dose	80 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Source	Strain Bor:NMRI (SPF Han), Breeder [redacted])
3.2.3	Sex	Male and female

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Section A6.6.5 Genotoxicity in vivo/ Second study

Annex Point IIA6.6.5

Mouse micronucleus study

3.2.4	Age/weight at study initiation	8-12 weeks/28-41 g
3.2.5	Number of animals per group	5m + 5f per sampling time
3.2.6	Control animals	Yes
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 80 mg as/kg bw
3.3.6	Total volume applied	10 ml/kg bw
3.3.7	Controls	Vehicle
3.3.8	Substance used as Positive Control	Cyclophophamide (monohydrate), 20 mg/kg bw
3.3.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	bone marrow
		Number of animals: 10/time point
		Number of cells: Approximately 1000 polychromatic erythrocytes /experimental animal were counted
		Time points: 6, 24, 48 h after treatment or other
		Type of cells erythrocytes in bone marrow
		Parameters: the number of normachromatic erythrocytes/1000 polychromatic, the number of erythrocytes showing micronuclei

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Animals treated with imidacloprid showed symptoms of toxicity (apathy, decreased motility, respiratory difficulties) for up to six hours after administration. All animals survived until the end of the study.

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Section A6.6.5 Genotoxicity in vivo/ Second study

Annex Point IIA6.6.5

Mouse micronucleus 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 84-23329 guideline, imidacloprid technical was tested for clastogenic effects in bone marrow with the micronucleus test on the mouse following a single oral administration of 80 mg/kg bw. Imidacloprid was suspended in 0.5 % aqueous Cremophor emulsion. Cyclophosphamide was used as positive control and administered in doses of 20 mg/kg bw. Administration volume was 10 mL/kg bw. Polychromatic erythrocytes were evaluated for micronuclei at 24 hrs, 48 hrs and 72 hrs postdose.
5.2	**Results and discussion**	Animals treated with imidacloprid showed symptoms of toxicity (apathy, decreased motility, respiratory difficulties) for up to six hours after administration. All animals survived until the end of the study. The ratio of polychromatic to normochromatic erythrocytes was not altered. No indications of a clastogenic effect were found. The results for the positive control indicated a clear clastogenic effect.
5.3	**Conclusion**	Imidacloprid is found to be non clastogenic in mice bone marrow *in vivo*.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/09
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	

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Section A6.6.6 Genotoxicity in vivo / Germ cell effects

Annex Point IIA6.6.6

Mouse germ-cell cytogenetic assay

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.4.3, II A, 5.4.3 /01*
	Authors (year)	[redacted] (1990)
	Title	Mouse germ-cell cytogenetic assay with NTN 33893
	Company, report No.	Bayer CropScience AG, Report-No.: R5063 BES Ref. : M-026551-01-1
	Date	1990-05-22
	Testing facility	[redacted]
	Dates of work	September 1989 – February 1990
	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		
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 483; EU 87/302EEC
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, batch no. 180587, purity: 94.1 %.
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	
3.1.2.3	Maximum tolerable dose	80 mg/kg bw
3.2	**Test Animals**	
3.2.1	Species	mouse
3.2.2	Strain	NMRI mice
3.2.3	Source	Breeder [redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	Minimum 10 weeks/approximately 30 g

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Section A6.6.6 Genotoxicity in vivo / Germ cell effects

Annex Point IIA6.6.6 *Mouse germ-cell cytogenetic assay*

3.2.6	Number of animals per group	6m per sampling time
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral for a.s. (i.p. for positive control)
3.3.1	Number of applications	1
3.3.2	Postexposure period	6, 24, 48 h after treatment or other
		Oral
3.3.3	Type	gavage
3.3.4	Concentration	80 mg/kg bw
3.3.5	Vehicle	0.5% cremaphor aqueous solution
3.3.6	Concentration in vehicle	Sufficient to deliver the equivalent of 80 mg/kg bw
3.3.7	Total volume applied	10 ml
3.3.8	Controls	Vehicle
3.3.9	Substance used as Positive Control	Doxorubicin-sulfate hydrochloride
3.4	**Examinations**	
3.4.1	Clinical signs	Not reported
3.4.2	Tissue	testes
		Number of animals: 6 animals/test group
		Number of cells: At least 100 metaphases/experimental animal were scored
		Time points: 6, 24, 48 h after treatment
		Parameters: Cytotoxicity, mitotic index
		4 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	The test article did not induce chromosome aberrations at the maximum tolerated dose of 80 mg/kg bw.
4.2	**Other**	The results for the positive control indicated a clear clastogenic effect.

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Section A6.6.6 Genotoxicity in vivo / Germ cell effects

Annex Point IIA6.6.6 *Mouse germ-cell cytogenetic assay*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study done according to OECD 483, EU 87/302EEC guidelines, imidacloprid technical was tested for chromosome aberration by means of the mouse germ-cell cytogenetic assay *in vivo* following a single oral dose of 80 mg/kg bw. Doxorubicin-sulfate hydrochloride was used as positive control and administered intraperitoneally at a single dose of 10 mg/kg bw. Administration volume was 10 mL/kg bw.
5.2	**Results and discussion**	The test article did not induce chromosome aberrations at the maximum tolerated dose of 80 mg/kg bw. The results for the positive control indicated a clear clastogenic effect..
5.3	**Conclusion**	Imidacloprid is found to be non-mutagenic in the mouse germ cell chromosome aberration assay.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/09
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	

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Section A6.7/01 **Carcinogenicity**

Section A6.7/02 *Chronic toxicity and carcinogenicity study in the rat*

Annex Point IIA6.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP Monograph B6.5.1, II A, 5.5.1 /01and 02*	
	Authors (year)	[redacted] (1991 a and b)	
	Title	a) NTN 33893 (proposed c.n.: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months) b) NTN 33893 (proposed common name: Imidacloprid) - Chronic toxicity and cancerogenicity studies on Wistar rats (administration in food over 24 months) - supplementary MTD study for two-year study T1025699	
	Company, report No.	Bayer CropScience AG, Report-No.: a) 19925 b) 20541 BES Ref. : a) M-027741-02-1 b) M-027135-01-1	
	Date	a) 1991-01-25 b) 1991-08-19	
	Testing facility	[redacted]	
	Dates of work	a) July 1987 – July 1989 b) September 1988 – September 1990	
	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			
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 453, FIFRA § 83-5, EU 88/302/EEC	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	No	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid, mixed batch no.180587, purity: 94.3 % - 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		

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Section A6.7/01 Carcinogenicity

Annex Point IIA6.7

Chronic toxicity and carcinogenicity study in the rat

3.2	**Test Animals**	
3.2.1	Species	Male and female Wistar rats (Strain Bor:WISW (SPF Cpb); Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	56-102 g males / 59-89 g females / 4-6 weeks old
3.2.6	Number of animals per group	50 m, 50f/dose group in main and supplemental study 10m, 10f/dose group as 12 month sacrifice in both studies
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	2 years
3.3.2	Frequency of exposure	Continual via diet
3.3.3	Postexposure period	All animals sacrificed at end of exposure period
3.3.4	**Oral**	
3.3.4.1	Type	Main study in diet at concentrations of 0, 100, 300 and 900 ppm for 24 months. In a supplement MTD study, groups of 50 male and female Wistar rats were administered imidacloprid at levels of 0 and 1800 ppm in their diet for 24 months. Mean consumption of imidacloprid per kg body weight per day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.
3.3.4.2	Concentration	
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	Yes, per OECD 453, FIFRA § 83-5, EU 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.4.1.1	Clinical signs	
3.4.1.2	Mortality	
3.4.2	Body weight	
3.4.3	Food consumption	
3.4.4	Water consumption	
3.4.5	Ophthalmoscopic examination	

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3.4.6	Haematology	
3.4.7	Urinalysis	
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	Yes, per OECD 453, FIFRA § 83-5, EU 88/302/EEC, no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.5.2	Gross and histopathology	
3.5.3	Other examinations	TSH, T3 and T4 levels
3.5.4	Statistics	Mann-Whitney + Wilcoxon 2-tailed U-test, variance analyses

4 RESULTS AND DISCUSSION

4.1	**Observations**		
4.1.1	Clinical signs	Appearance, behaviour, food intakes and mortality were unaffected in males and females at 1800 ppm. Water intake was reduced by 13 % in females at 1800 ppm.	
4.1.2	Mortality		
4.2	**Body weight gain**	See Figures A6.7/01 & /02-1 to A6.7/01 & /02-4. Reduced weight gains were noted in males and females at 900 ppm and above with the decreases amounting to 11 – 12 % at 1800 ppm. The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight.	X
4.3	**Food consumption and compound intake**	No treatment related effects on food consumption noted. Mean consumption of imidacloprid per kg body weight and day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.	
4.4	**Ophtalmoscopic examination**	No treatment related effects noted.	
4.5	**Blood analysis**		
4.5.1	Haematology	The haematological tests gave no indications of haematotoxicity or damage to the haematogenic organs at dose levels up to 1800 ppm. The plasma, erythrocyte and brain cholinesterase activities were not significantly affected; adverse effects on, orfunctional impairment of any organ could not detected in males and females up to and including 1800 ppm.	
4.5.2	Clinical chemistry		
4.5.3	Urinalysis		

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Section A6.7/01 **Carcinogenicity**

Section A6.7/02 *Chronic toxicity and carcinogenicity study in the rat*

Annex Point IIA6.7

4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Absolute liver and kidney weights were reduced after 12 months in females at 900 ppm; males exhibited lower liver weights at 900 ppm at this time. These deviations from the control values are not attributed to liver or kidney damage, but are seen in relation to the reduced body weight gain in these dose groups (see Table A6.7/01 & /02-1).	X
4.6.2	Gross and histopathology	Histopathological assessment of these organs produced no evidence for treatment-related lesions (see Table A6.7/01 & /02-2). Increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in males beginning at 300 ppm and in females at 900 ppm. At 1800 ppm fewer colloid aggregations and more parafollicular hyperplasias of minimal intensity were observed. These findings occur spontaneously in ageing rats and indicate involution of isolated follicles related to senescence. In these studies they are regarded as a treatment effect on the thyroid resulting in premature biological ageing processes in this organ.	
4.7	**Other**	No treatment related changes in TSH, T3 or T4 were observed after 76 weeks of treatment at 1800 ppm.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a combined chronic toxicity oncogenicity study done according to OECD 453, FIFRA § 83-5, EU 88/302/EEC guidelines, imidacloprid was administered to groups of 50 male and 50 female Wistar rats in their diet at concentrations of 0, 100, 300 and 900 ppm for 24 months. In a supplement MTD study, groups of 50 male and female Wistar rats were administered imidacloprid at levels of 0 and 1800 ppm in their diet for 24 months. Ten additional rats per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was: 5.7, 16.9, 51.3 and 102.6 mg for males and 7.6, 24.9, 73.0 and 143.7 mg for females.

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Section A6.7/01 **Carcinogenicity**

Section A6.7/02 *Chronic toxicity and carcinogenicity study in the rat*

Annex Point IIA6.7

5.2	**Results and discussion**	Appearance, behaviour, food intakes and mortality were unaffected in males and females at 1800 ppm. Water intake was reduced by 13 % in females at 1800 ppm.	
		Reduced weight gains were noted in males and females at 900 ppm and above with the decreases amounting to 11 – 12 % at 1800 ppm. The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight.	X
		The haematological tests gave no indications of haematotoxicity or damage to the haematogenic organs at dose levels up to 1800 ppm. The plasma, erythrocyte and brain cholinesterase activities were not significantly affected; adverse effects on, or functional impairment of any organ could not detected in males and females up to and including 1800 ppm.	
		Absolute liver and kidney weights were reduced after 12 months in females at 900 ppm; males exhibited lower liver weights at 900 ppm at this time. These deviations from the control values are not attributed to liver or kidney damage, but are seen in relation to the reduced body weight gain in these dose groups.	X
		Histopathological assessment of these organs produced no evidence for treatment-related lesions. Increased incidence of mineralisation in the colloid of the thyroid gland follicles was observed in males beginning at 300 ppm and in females at 900 ppm. At 1800 ppm fewer colloid aggregations and more parafollicular hyperplasias of minimal intensity were observed. These findings occur spontaneously in ageing rats and indicate involution of isolated follicles related to senescence. In these studies they are regarded as a treatment effect on the thyroid resulting in premature biological ageing processes in this organ. No treatment related changes in TSH, T3 or T4 were observed after 76 weeks of treatment at 1800 ppm.	
		The nature, location, incidence and latency periods of the tumors in this study presented no evidence for an oncogenic effect of imidacloprid.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	300 ppm and 900 ppm in males and females, respectively , based on thyroid effects (increased incidence of colloid mineralisation) and on reduced body weight gains at 900 ppm in both sexes	
5.3.2	NO(A)EL	100/300 ppm (males/females), equivalent of 5.7 mg/kg bw/day for males and 24.9 mg/kg bw/day for females	
5.3.3	Other	The nature, location, incidence and latency periods of the tumors in this study presented no evidence for an oncogenic effect of imidacloprid.	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	X

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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	4.2/5.2 Reduced weight gain was noted in females at 1800 ppm (-12 %). The 1800 ppm level is regarded to be the maximum tolerated dose due to the significant reduction in the body weight of more than 10 % (cf. CA-Table 1). 4.6.1/5.2 Absolute liver, kidney, adrenal, heart, and spleen weights were significantly reduced after 24 months in females at 1800 ppm; males exhibited lower liver weights at 900 ppm after 12 months. Except for the liver weight at 1800 ppm in females after 24 months, relative organ weights do not differ statistically from the control values. Thus, the reduced organ weights are considered not to be attributed to organ damage, but are seen in relation to the reduced body weight gain in these dose groups at the specified times (see Table A6.5/01 & /02-1, CA-Table 2).
Conclusion	Applicant's version is acceptable: LOAEL: 17/73 mg/kg bw/d (M/F) based on mineralisation in the colloid of the thyroid gland follicles. NOAEL: 6/25 mg/kg bw/d (M/F)
Reliability	1
Acceptability	Acceptable
Remarks	2.3/5.3.5 Deficiencies: 10 instead of 20 animals in the satellite groups for interim sacrifice. The deviation does not affect the overall validity of the study.
	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	

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Figure A6.7/01 & /02-1: Main chronic study in rats - Body weights (mean values) males

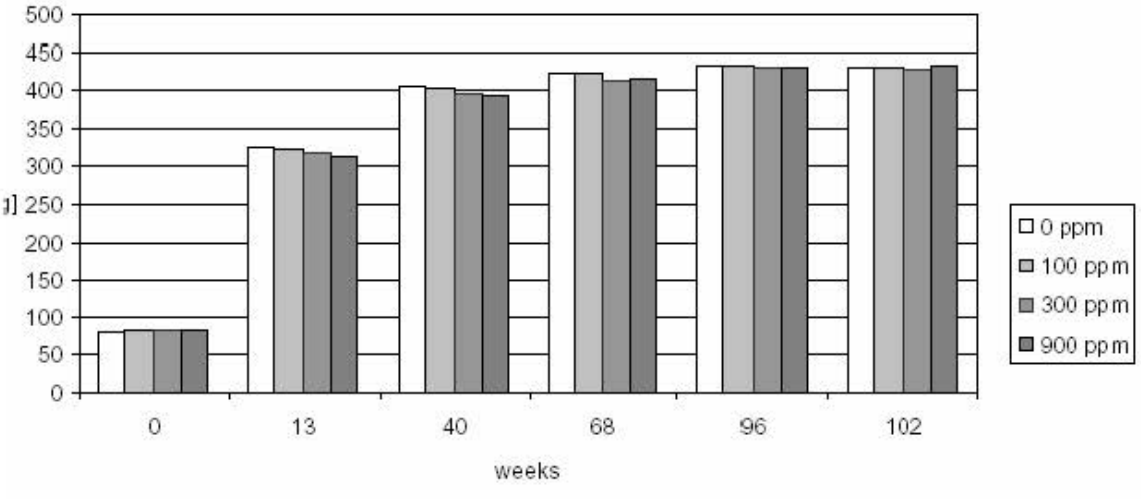

Figure A6.7/01 & /02-2: Supplement chronic study in rats - Body weights (mean values) males

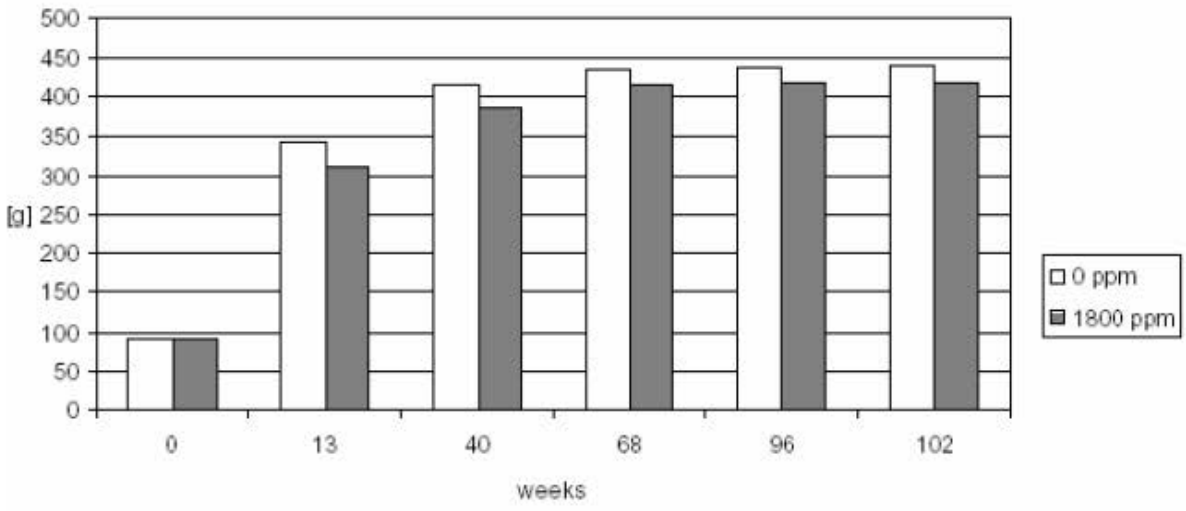

Figure A6.7/01 & /02-3: Main chronic study in rats - Body weights (mean values) females

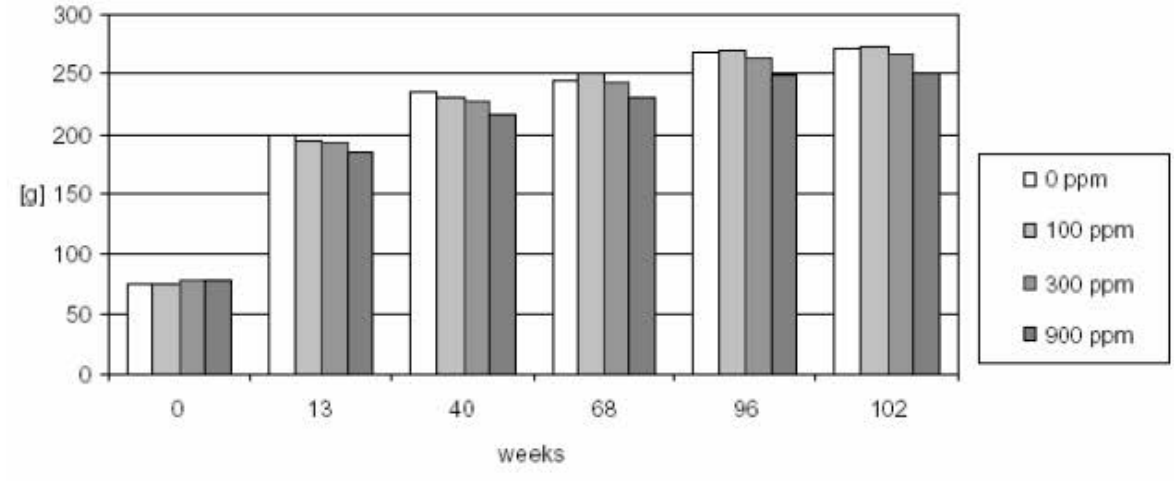

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Figure A6.7/01 & /02-4: Supplement chronic study in rats - Body weights (mean values) females

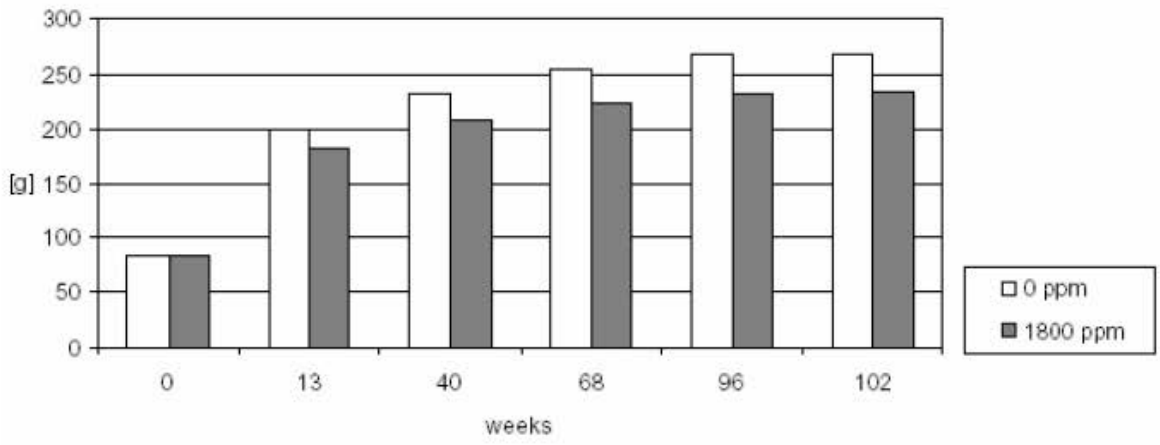

Table A6.7/01 & /02-1. Chronic study in rats-Absolute organ weights after 12 months

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

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

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Table A6.7/01 & /02-2. Chronic study in rats-Histopathological findings

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

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Section A6.7/03 Carcinogenicity

Section A6.7/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.7.2

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1 REFERENCE

1.1	**Reference**	*PPP Monograph B6.5.2, II A, 5.5.2 /01and 02*
	Authors (year)	[redacted] (1991 a and b)
	Title	a) NTN 33893 (proposed common name Imidacloprid) - Carcinogenicity study on B6C3F1 mice (administration in the food for 24 months) b) NTN 33893 (proposed common name: Imidacloprid) - Carcinogenicity study in B6C3F1 mice (supplementary MTD testing for study T5025710 with administration in diet over a 24-month period)
	Company, report No.	Bayer CropScience AG, Report-No.: a) 19931 b) 20769 BES Ref. : a) M-026310-01-1 b) M-026038-01-1
	Date	a) 1991-01-28 b) 1991-10-24
	Testing facility	[redacted]
	Dates of work	a) September 1987 – September 1989 b) September 1988 – September 1990
	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		
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 compliance with OECD 451, FIFRA, § 83-2.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, mixed batch no.180587, purity: 95.3 %; supplementary study: 95.0 %
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	

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Section A6.7/03 Carcinogenicity

Section A6.7/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.7.2

3.2	**Test Animals**	
3.2.1	Species	Male and female B6C3F1 mice (Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	18-25 g males / 14-18 g females / 5 weeks old
3.2.6	Number of animals per group	50 m, 50f/dose group in main and supplemental study 10m, 10f/dose group as 12 month sacrifice in both studies
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	24 months
3.3.2	Frequency of exposure	Continual in diet
3.3.3	Postexposure period	All animals sacrificed at the end of the exposure period
3.3.4	**Oral**	
3.3.4.1	Type	Main study in the diet at concentrations of 0, 100, 330 and 1000 ppm
3.3.4.2	Concentration	In a supplement MTD study, groups of 50 male and female mice were administered imidacloprid at levels of 0 and 2000 ppm in their diet for 24 months. Mean consumption of imidacloprid per kg body weight per day was 20.2, 65.6, 208.2, or 413.5 mg for males and 30.3, 103.6, 274.4, or 423.9 mg for females.
3.3.4.3	Vehicle	
3.3.4.4	Concentration in vehicle	
3.3.4.5	Total volume applied	
3.3.4.6	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	Yes, per OECD 451, FIFRA, § 83-2, no deviations noted by the RMS in the December 2005 91/414 draft DAR
3.4.1.1	Clinical signs	
3.4.1.2	Mortality	
3.4.2	Body weight	
3.4.3	Food consumption	
3.4.4	Water consumption	

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Section A6.7/03 **Carcinogenicity**

Section A6.7/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.7.2

3.4.5	Ophthalmoscopic examination		
3.4.6	Haematology		
3.4.7	Clinical Chemisty		
3.4.8	Urinalysis		
3.5	**Sacrifice and pathology**		
3.5.1	Organ Weights	Yes, per OECD 451, FIFRA, § 83-2, no deviations noted by the RMS in the December 2005 91/414 draft DAR	
3.5.2	Gross and histopathology		
3.5.3	Other examinations		
3.5.4	Statistics	Mann-Whitney & Wilcoxon 2-tailed U-test, generalized Wilcoxin test, generalized Kuskal-Wallis test	
		4 RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	Unusual vocalisations, squeaking and twittering, which increased whenever the animals became agitated were observed throughout the study in males and females at 2000 ppm. In addition, hypersensitivity to ether narcosis and/or blood withdrawal resulting in increased mortality after manipulations was observed in this dose group. Nine males and four females died after anaesthesia for tattooing or blood sampling compared with no mortalities in the control group. Similar observations were made in the 15-week range-finding study (non-key study).	X
4.1.2	Mortality		
4.2	**Body weight gain**	See Table A6.7/03 & /04-1	
4.3	**Food consumption and compound intake**	Food intake was slightly reduced in females at 1000 ppm and markedly reduced in females (-24 %) at 2000 ppm. Water intake was decreased slightly in females at 1000 ppm and markedly in males (-11 %) and females (-27 %) at 2000 ppm. See Table A6.7/03 & /04-2.	
		Body weight development was not influenced at doses up to and including 330 ppm. At 1000 ppm the mice exhibited reduced weight gain and marked reductions were seen at 2000 ppm (up to -29 % in males and -26 % in females).	X
4.4	**Ophtalmoscopic examination**	No treatment related effects noted.	
4.5	**Blood analysis**		
4.5.1	Haematology	No indications of treatment-related haemotoxicity or damage to the haematogenic organs was found at 1000 ppm. At 2000 ppm lower leukocyte counts were determined in both sexes. The clinical chemistry gave no evidence for liver damage. Reduced blood cholesterol levels at 2000 ppm indicate an effect on the lipid metabolism in this group. Kidney related clinical chemistry parameters in the blood were unaffected in the 2000 ppm group. See Table A6.7/03 & /04-3.	X
4.5.2	Clinical chemistry		
4.5.3	Urinalysis		

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Section A6.7/03 **Carcinogenicity**

Section A6.7/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.7.2

4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	Morphological evidence for a marginal functional effect on the liver (low-grade periacinal hepatic cell hypertrophy) was found in a few males at 2000 ppm. This marginal change probably results from hepatocyte adaptation to the foreign substance metabolism, and should not be interpreted as evidence for liver damage. At 2000 ppm more animals than in the control groups exhibited mineralisation of the thalamic region of the brain. The region of brain mineralisation is only identified in the report on the MTD study but not in the main study, so that a direct comparison with the findings of the first study is not possible (see Table A6.7/03 & /04-4).	X
4.6.2	Gross and histopathology		
4.7	**Other**	The nature, location, incidence and latency periods of the detected tumors presented no evidence for an oncogenic effect.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an oncogenicity study done in complance with OECD 451, FIFRA, § 83-2 guidelines, imidacloprid was administered to groups of 50 male and 50 female mice in their diet at concentrations of 0, 100, 330 and 1000 ppm for 24 months. In a supplement MTD study, groups of 50 male and female mice were administered imidacloprid at levels of 0 and 2000 ppm in their diet for 24 months. Ten additional mice per sex and dose were included in both studies for an interim sacrifice after 12 months of treatment. Mean consumption of imidacloprid per kg body weight and day was 20.2, 65.6, 208.2, or 413.5 mg for males and 30.3, 103.6, 274.4, or 423.9 mg for females.	
5.2	**Results and discussion**	Unusual vocalisations, squeaking and twittering, which increased whenever the animals became agitated, were observed throughout the study in males and females at 2000 ppm. In addition, hypersensitivity to ether narcosis and/or blood withdrawal resulting in increased mortality after manipulations was observed in this dose group. Nine males and four females died after anaesthesia for tattooing or blood sampling compared with no mortalities in the control group.	X
		Food intake was slightly reduced in females at 1000 ppm and markedly reduced in females (-24 %) at 2000 ppm. Water intake was decreased slightly in females at 1000 ppm and markedly in males (-11 %) and females (-27 %) at 2000 ppm. Body weight development was not influenced at doses up to and including 330 ppm. At 1000 ppm the mice exhibited reduced weight gain and marked reductions were seen at 2000 ppm (up to -29 % in males and -26 % in females).	
		No indications of treatment-related haemotoxicity or damage to the haematogenic organs was found at 1000 ppm. At 2000 ppm lower leukocyte counts were determined in both sexes. The clinical chemistry gave no evidence for liver damage. Reduced blood cholesterol levels at 2000 ppm indicate an effect on the lipid metabolism in this group. Kidney related clinical chemistry parameters in the blood were unaffected in the 2000 ppm group.	

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Section A6.7/03 **Carcinogenicity**

Section A6.7/04 *Chronic toxicity and carcinogenicity study in the mouse*

Annex Point IIA6.7.2

		Morphological evidence for a marginal functional effect on the liver (low-grade periacinal hepatic cell hypertrophy) was found in a few males at 2000 ppm. This marginal change probably results from hepatocyte adaptation to the foreign substance metabolism, and should not be interpreted as evidence for liver damage. At 2000 ppm more animals than in the control groups exhibited mineralisation of the thalamic region of the brain. The region of brain mineralisation is only identified in the report on the MTD study but not in the main study, so that a direct comparison with the findings of the first study is not possible	
5.3	**Conclusion**		
5.3.1	LO(A)EL	1000 ppm in males and females, respectively , based on reduced body weights	X
5.3.2	NO(A)EL	330 ppm (males/females), equivalent to 65.5 mg/kg bw/day for males and 103.6 mg/kg bw/day for females	X
5.3.3	Other	The nature, location, incidence and latency periods of the detected tumors presented no evidence for an oncogenic effect.	
5.3.4	Reliability	1	
5.3.5	Deficiencies	No	

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/02/06
Materials and Methods	Applicant's version is acceptable.
Results and discussion	4.1.2/5.2 Nine males and four females in the treated groups died due to an increased sensitivity to ether anaesthesia for tattooing or blood sampling compared with one mortality in the control group. Despite the fact that marking animals by tattooing only 6 wk after study begin appears unusual, it is noted that this indirectly substance-related mortality was significantly increased in high-dose males whereas for females, no significance was obtained. However, this is obviously due to an unusually high early mortality rate in the female control group (4 dead animals by study wk 12), the reasons for which were not clearly explained in the study report. 4.2/5.2 Body weight development was not markedly (< 10 %) influenced at doses up to and including 1000 ppm. At 2000 ppm, body weight gain was decreased up to 25 % in males and up to 20 % in females (see CA-Table 1). 4.5.2/5.2 Significant elevation of AP in the 2000 ppm group is indicative for liver toxicity (see CA-Table 2). 4.6.1/5.2 Absolute and relative liver weights were significantly decreased in females at 2000 ppm. At 2000 ppm, more animals than in the control groups exhibited mineralisation of the thalamic region of the brain, however, the number of animals affected did not differ significantly from the control group.
Conclusion	Different from the applicant's version, the LOAEL and NOAEL are as follows: LOAEL: 414/424 mg/kg bw/d (M/F, 2000 ppm) based on markedly (> 10 %) decreased body weight, liver effects (hepatocyte hypertrophy, decreased liver weight and elevated AP) and decreased cholesterol NOAEL: 208/274 mg/kg bw/d (M/F, 1000 ppm)
Reliability	1
Acceptability	Acceptable (without restrictions for the endpoint carcinogenicity)
Remarks	
	COMMENTS FROM ... *(specify)*
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	

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Table A6.7/03 & /04-1. Chronic study in mice-Mean body weights

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

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

Table A6.7/03 & /04-2. Supplement chronic study in mice-Food and water intake

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

Table A6.7/03 & /04-3. Chronic study in mice-Haematology and clinical chemistry

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

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

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Table A6.7/03 & /04-4. Chronic study in mice-Histopathological findings

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

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Section A6.8.1/01 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rats dosed by oral gavage

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.6.2.1, II A, 5.6.2.1 /01*
	Authors (year)	[redacted] (1988a)
	Title	Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rat
	Company, report No.	Bayer CropScience AG, Report-No.: R5442 BES Ref. : M-027900-04-1
	Date	24.11.1988, Amended 03.03.1992
	Testing facility	[redacted]
	Dates of work	July – August 1987
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 17001/87)
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 414; EPA FIFRA §83-3
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Imidacloprid, batch no. PT. 17001/87, purity: 94.2 %
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	Female Wistar/HAN rats (Strain Kfm:WIST; Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	

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Section A6.8.1/01 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rats dosed by oral gavage

3.2.5	Age/weight at study initiation	184 to 240 g females / 11 weeks old minimum
3.2.6	Number of animals per group	25
3.2.7	Control animals	yes
3.2.8	Mating period	Per OECD 414; EPA FIFRA §83-3, no deviations noted by the RMS in the December 2005 91/414 DAR.
3.3	**Administration/ Exposure**	
3.3.1	Duration of exposure	Imidacloprid was administered to groups of 25 mated female Wistar rats orally by gavage from day 6 through to day 15 post coitum at doses of 0 (vehicle only) , 10, 30 or 100 mg/kg bw/day in 0.5 % aqueous Cremophor suspension. A standard dose volume of 10 mL/kg bw with daily adjustment to the actual body weight was used.
3.3.2	Type	
3.3.3	Concentration	
3.3.4	Vehicle	Females were sacrificed on day 21 p.c. and the foetuses were removed by Caesarean section
3.3.5	Concentration in vehicle	
3.3.6	Total volume applied	
3.3.7	Controls	
3.4	**Examinations**	
3.4.1	Body weight	Per OECD 414; EPA FIFRA §83-3, no deviations noted by the RMS of the December 2005 draft 91/414 DAR
3.4.2	Food consumption	
3.4.3	Clinical signs	
3.4.4	Examination of uterine content	
3.4.5	Examination of foetuses	
3.4.5.1	General	Per OECD 414; EPA FIFRA §83-3, no deviations noted by the RMS of the December 2005 draft 91/414 DAR
3.4.5.2	Skeletal	
3.4.5.3	Soft tissue	

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Section A6.8.1/01 **Teratogenicity Study**

Annex Point IIA6.8.1 *Teratogenicity in rats dosed by oral gavage*

4 RESULTS AND DISCUSSION

4.1 Maternal toxic Effects

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

At the highest dose tested no treatment-related changes were observed in the reproduction parameters (incidence of pregnant females and of females with viable foetuses, rates of implantation, viable foetuses, resorptions, mean foetal weights per litter, ratio of male to female foetuses). See Table A.6.8.1/01-1 for maternal data.

4.2 Teratogenic / embryotoxic effects

No treatment-related changes were determined from external and visceral examination of the foetuses. In the skeletal examination, a slightly increased incidence of wavy ribs (reversible alteration in shape) was observed at 100 mg/kg bw/day. Thus, embryotoxicity of imidacloprid is observed only at a dose which induces moderate maternal toxicity. See Table A.6.8.1/01-2 for litter data.

4.3 Other effects

None noted

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a teratogenicity study conducted according to OECD 414, EPA FIFRA §83-3 guidelines, imidacloprid was administered to groups of 25 mated female Wistar rats orally by gavage from day 6 through to day 15 post coitum at doses of 0, 10, 30 or 100 mg/kg bw/day in 0.5 % aqueous Cremophor suspension. A standard dose volume of 10 mL/kg bw with daily adjustment to the actual body weight was used. Females were sacrificed on day 21 p.c. and the foetuses were removed by Caesarean section for appropriate examinations.

5.2 Results and discussion

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

At the highest dose tested no treatment-related changes were observed in the reproduction parameters (incidence of pregnant females and of females with viable foetuses, rates of implantation, viable foetuses, resorptions, mean foetal weights per litter, ratio of male to female foetuses).

No treatment-related changes were determined from external and visceral examination of the foetuses. In the skeletal examination, a slightly increased incidence of wavy ribs (reversible alteration in shape) was observed at 100 mg/kg bw/day. Thus, embryotoxicity of imidacloprid is observed only at a dose which induces moderate maternal toxicity.

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Section A6.8.1/01 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rats dosed by oral gavage

5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	100 mg/kg bw/day based on reduced body weight gain and reduced food consumption
5.3.2	NO(A)EL maternal toxic effects	30 mg/kg bw/day
5.3.3	LO(A)EL embryotoxic / teratogenic effects	100 mg/kg bw/day based on increased incidence of wavy ribs
5.3.4	NO(A)EL embryotoxic / teratogenic effects	30 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

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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	4.2 *In the view of the RMS, wavy ribs constitute a slight, transient alteration, which is fully reversed if pups are raised to age. This has a. o. been documented in the following publications:* ▪ *Kast, A. (1994): "Wavy ribs". A reversible pathologic finding in rat fetuses. Exp. Toxicol. Pathol. 46: 203-210* ▪ *Khera, K. (1981): Common fetal aberrations and their teratologic significance: a review. Fund. Appl. Toxicol. 1: 13-18* ▪ *Nishimura, M.; Iizuka, M.; Iwaki, S. (1982): Repairability of drug-induced "wavy ribs" in rat offspring. Armifo 32: 1515-1522* *The occurrence of wavy ribs should therefore not be rated as an adverse effect. Consequently, the developmental NOAEL in the rat teratogenicity study in rats is set at 100, not at 30 mg/kg bw/d.*
Conclusion	5.2 Cf. 4.2. The developmental NOAEL in this study is 100 mg/kg bw/d (LOAEL > 100 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	

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Table A6.8.1/01-1: Rat developmental toxicity: Maternal effects

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

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

Table A6.8.1 /01-2: Rat developmental toxicity: Litter effects

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

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Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rabbits dosed by oral gavage

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.6.6.2.1, II A, 5.6.2.1 /01*
	Authors (year)	[redacted] (1988b)
	Title	Embryotoxicity study (including teratogenicity) with NTN 33893 technical in the rabbit
	Company, report No.	Bayer CropScience AG, Report-No.: R5443 BES Ref. : M-027920-04-1
	Date	24.11.1988, Amended 03.03.1992
	Testing facility	[redacted]
	Dates of work	June – July 1987
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 17001/87)
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 414; EPA FIFRA §83-3.
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	
3.1.1	Lot/Batch number	Imidacloprid, batch no. PT. 17001/87, purity: 94.2 %
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	Female chinchilla rabbits (Strain CHbb:CH hybrids; Breeder [redacted])
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	

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Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rabbits dosed by oral gavage

3.2.5	Age/weight at study initiation	2650 to 4064 grams / 4 to 6 months old	
3.2.6	Number of animals per group	16	
3.2.7	Control animals	yes	
3.2.8	Mating period	Per OECD 414; EPA FIFRA §83-3, no deviations noted by the RMS in the December 2005 91/414 DAR.	
3.3	**Administration/ Exposure**		
3.3.1	Duration of exposure	Imidacloprid was administered to groups of 16 female chinchilla rabbits orally by gavage at doses of 0 (vehicle only), 8, 24 or 72 mg/kg bw/day from day 6 through to day 18 p.c. in 0.5 % aqueous Cremophor suspension. A standard volume of 4 mL/kg bw with daily adjustment to the actual body weight was used. Females were sacrificed on day 28 p.c. and the foetuses were removed by Caesarean section.	
3.3.2	Type		
3.3.3	Concentration		
3.3.4	Vehicle		
3.3.5	Concentration in vehicle		
3.3.6	Total volume applied		
3.3.7	Controls		
3.4	**Examinations**		
3.4.1	Body weight	Per OECD 414; EPA FIFRA §83-3, with one deviation noted by the RMS of the December 2005 draft 91/414 DAR-no examination of foetal heads for skeletal changes	X
3.4.2	Food consumption		
3.4.3	Clinical signs		
3.4.4	Examination of uterine content		
3.4.5	Examination of foetuses		

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Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rabbits dosed by oral gavage

3.4.5.1	General	Per OECD 414; EPA FIFRA §83-3, with one deviation noted by the RMS of the December 2005 draft 91/414 DAR-no examination of foetal heads for skeletal changes	
3.4.5.2	Skeletal		
3.4.5.3	Soft tissue		
		4 RESULTS AND DISCUSSION	
4.1	**Maternal toxic Effects**	Decreased body weight gains were found at 24 mg/kg bw/day and higher. Body weight loss from the start of treatment until day 20 p.c. decreased food consumption during the treatment period and mortality were observed at 72 mg/kg bw/day. Two females died on days 18 and 19 p.c., at the end of the treatment period. A further female from this group aborted on day 26 post coitum and two females showed total litter resorption at terminal necropsy. See Table A.6.8.1/02-1 for maternal data.	X
4.2	**Teratogenic / embryotoxic effects**	The body weights of the foetuses were slightly reduced (although the difference did not reach statistical significance) and the incidence of foetuses with retarded ossification was increased at 72 mg/kg bw/day. Because of the reduced litter size in this group which would have resulted in increased foetal weights had there not been foetal toxicity, the reduced foetal weights and the skeletal changes are regarded as signs of foetal retardation and may have resulted from the severe maternal toxicity. No treatment-related malformation were observed. See Table A.6.8.1/02-2 for litter data.	X
4.3	**Other effects**	None noted	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In a teratogenicity study conducted according to OECD 414; EPA FIFRA §83-3 guidelines, imidacloprid was administered to groups of 16 female chinchilla rabbits orally by gavage at doses of 0, 8, 24 or 72 mg/kg bw/day from day 6 through to day 18 p.c. in 0.5 % aqueous Cremophor suspension. A standard volume of 4 mL/kg bw with daily adjustment to the actual body weight was used. Females were sacrificed on day 28 p.c. and the foetuses were removed by Caesarean section for appropriate examinations.	
5.2	**Results and discussion**	Decreased body weight gains were found in dams at 24 mg/kg bw/day and higher. Body weight loss from the start of treatment until day 20 p.c. decreased food consumption during the treatment period and mortality were observed at 72 mg/kg bw/day. Two females died on days 18 and 19 p.c., at the end of the treatment period. A further female from this group aborted on day 26 post coitum and two females showed total litter resorption at terminal necropsy. The body weights of the foetuses were slightly reduced (although the difference did not reach statistical significance) and the incidence of foetuses with retarded ossification was increased at 72 mg/kg bw/day. Because of the reduced litter size in this group which would have resulted in increased foetal weights had there not been foetal toxicity, the reduced foetal weights and the skeletal changes are regarded as signs of foetal retardation and may have resulted from the severe maternal toxicity. No treatment-related malformations were observed.	X X

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Section A6.8.1/02 Teratogenicity Study

Annex Point IIA6.8.1

Teratogenicity in rabbits dosed by oral gavage

5.3	**Conclusion**	
5.3.1	LO(A)EL maternal toxic effects	24 mg/kg bw/day based on reduced body weight gain
5.3.2	NO(A)EL maternal toxic effects	8 mg/kg bw/day
5.3.3	LO(A)EL embryotoxic / teratogenic effects	72 mg/kg bw/day based on reduced body weights of foetuses and retarded ossification
5.3.4	NO(A)EL embryotoxic / teratogenic effects	24 mg/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	no examination of foetal heads for skeletal changes

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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	4.1/5.2 Body weight of the dams was significantly and markedly decreased at 72 mg/kg bw/d (see CA-Table 1). 4.2/5.2 No treatment-related malformations were observed. For details of the observed abnormalities, see CA-Table 2. Heading of Table A6.8.1.1/02-2: Rat developmental toxicity - Litter effects should read: Rabbit developmental toxicity - Litter effects Table A6.8.1.1/02-2: Highest dose level was 72, not 74 mg/kg bw/d
Conclusion	Differing from the applicant's version the NOAEL/LOAEL are as follows: NOAEL(maternal): 24 mg/kg bw/d based on reduced body weight gain at : 72 mg/kg bw/d N.b.: During the peer review under Dir. 91/414/EEC, the PRAPeR meeting established a maternal NOAEL of 8 mg/kg bw/d based on reduced body weight gain in dams from day 6-16 p.c. The results for maternal toxicity observed in this study are presented in Table A6.8.1/02-1. In the opinion of the RMS, the apparent effect on body weight gain should not be considered for risk assessment as a) terminal body weight was only slightly affected (< 10 %) b) and so was corrected weight gain (i. e. body weight gain excluding the gravid uterus). NOAEL(embryotoxicity/teratogenicity): 24 mg/kg bw/d based on reduced foetal body weight and retarded ossification at 72 mg/kg bw/d
Reliability	2
Acceptability	Acceptable with restrictions; 3.4 deviation from OECD 414 in that heads were not examined for skeletal alterations
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*

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Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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