Section 7.4.3.2/01 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII 2.2

Early life stage study on Oncorhynchus mykiss

from 8.9 to 11.5 °C. Four out of 196 temperature measurements were out of the specified range with a maximum of 11.3 °C. It is the opinion of the study director and the RMS of the December 2005 91/414 DAR that this deviation has no impact on the general outcome and validity of the study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/13
Materials and Methods	Applicant's version is acceptable with the following comments: 3.4.3: 200 eggs (4 egg incubation cups with 50 eggs each) were distributed to each replicate. After completion of hatch, 30 randomly selected larvae were transferred into laval chambers.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.4.3.2/01-1: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*
Source	Forellenhof Mandli, 4410 Liestal, Switzerland
Wild caught	No
Age/size	Purchased as fertilized eggs
Kind of food	Fry were fed live brine shrimp starting day 42
Amount of food	*Ad libitum*
Feeding frequency	At least twice daily
Post-hatch transfer time	Day 0 to larval chambers, day 14 to test vessaels
Time to first feeding	Day 42
Feeding of animals during test	Yes except for 24 hours prior to sacrifice
Treatment for disease within 2 weeks preceeding test	Not noted

Table A7.4.3.2/01-2 Cumulative mortality data for rainbow trout when exposed to imidacloprid

Test-Substance Concentration measured [mg/l]	Cumulative mortality (C.M.) on day after start of exposure (replicates A/B): number of fish										
	37-41	42	43	44	45-46	47-51	52-54	55	56-71	71-91	Mean %C.M.*
Control	0/0	0/0	0/0	0/0	0/0	1/0	1/0	1/0	1/0	2/0	3.3
0.0994	0/0	0/1	0/1	0/2	1/2	1/2	1/2	1/2	1/2	1/2	5.0
0.307	0/0	0/0	0/0	0/3	1/3	1/3	1/3	2/3	3/3	3/3	10.0
0.977	0/0	0/1	0/1	0/1	0/1	0/2	0/2	1/2	1/2	1/2	5.0
3.14	0/0	0/0	0/0	0/0	0/0	0/0	1/0	1/0	1/0	1/0	1.7
9.02	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
26.9	0/0	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	3.3

***mean % cumulative mortality over 91 days**

Table A7.4.3.2/01-3 Early life-stage toxicity of imidacloprid on Rainbow trout

Endpoints		Concentration		Concentration
Time to hatch	NOEC:	9.02 mg as/L	LOEC:	26.9 mg as/L
Hatching rate (study day 31)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Larval deformities (study day 45)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Larval survival (study day 45)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Time to swim-up on d 40, 42–49	NOEC:	9.02 mg as/L	LOEC:	26.9 mg as/L
Swim-up on day 52 (control swim-up = 95 %)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Behavioural changes	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Post hatch survival (study day 91)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Length (study day 91)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Wet weight (study day 91)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Dry weight (study day 91)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L
Deformations at the end of exposure (visual assessment)	NOEC:	= 26.9 mg as/L	LOEC:	> 26.9 mg as/L

Table A7_4_3_2-6: Validity criteria for fish tests according to OECD Guidelines 210/212

	fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	X	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	X	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	X	
Test substance concentrations maintained within ± 20% of mean measured values	X	
No effect on survival nor any other adverse effect found in control	X	

Section 7.4.3.4/01
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Chronic static renewal toxicity to Daphnia magna

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2.4, II A, 8.2.5 /01*
	Authors (year)	Young, B. M.; Blakemore, G. C. (1990)
	Title	21-day chronic static renewal toxicity of NTN 33893 to Daphnia magna
	Company, report No.	Bayer CropScience AG, Report-No.: 100247 BES Ref. : M-006824-01-1
	Date	1990-09-19
	Testing facility	[redacted]
	Dates of work	June 1, 1990 – June 22, 1990
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 9030211)
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**	US- EPA-FIFRA 72-4
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893: purity: 95.4 % (Lot No.: 9030211)
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	HPLC with UV detection, validated by ABC laboratories in ABC report 37859

Section 7.4.3.4/01 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

Chronic static renewal toxicity to Daphnia magna

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant, not poorly soluble	
3.3	**Reference substance**	No	
3.4	**Testing procedure**		x
3.4.1	Dilution water	Study conducted in accordance with U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.2	Test organisms	6 first instars of *Daphnia magna* (see Table A7.4.3.4/01-1)	
3.4.3	Handling of offspring	Study conducted in accordance U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.4	Test system		
3.4.5	Test conditions		
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Water quality parameters (temperature, pH, oxygen) were measured weekly	
3.4.8	Examination / Sampling	Biological observations according to guideline; a brief summary: Daily, survival, abnormal effects and observance of first brood; reproductive success by counting offspring produced in eachconcentration 3 times weekly (M,W,F)	
3.4.9	Monitoring of TS concentration	Yes, on days 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21	
3.4.10	Statistics	One-tailed Fisher's exact, t-Test, Dunnett's one-tailed multiple mean comparison, ANOVA, Shapiro-Wilk normality	
		4 RESULTS	
4.1	**Range finding test**	Performed	
4.1.1	Concentrations	1.2, 2.5, 5.0 and 10 mg a.s./L	
4.1.2	Number/ percentage of animals showing adverse effects	Over 16 days, no significant mortality at highest dose. Daphnids in 10 mg/L group were smaller than other groups.	
4.1.3	Nature of adverse effects	Total instar production lower at 5.0 and 10.0 mg/L: 787(control), 656 (solvent control), 717 (0.6 mg/L), 699 (1.2 mg/L), 566 (2.5 mg/L), 336 (5.0 mg/L), 11 (10.0 mg/L)	
4.2	**Results test substance**		

Section 7.4.3.4/01 Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Chronic static renewal toxicity to Daphnia magna

4.2.1	Initial concentrations of test substance	Nominal concentrations of 0 (control), 0.49, 0.94, 1.9, 3.8 and 7.5 mg a.s./L. Except at 1 time point (day 5) in the 0.94 mg/L system, all measured concentrations were between 80 and 105% of nominal at all time points across all concentrations. Mean measured concentrations were 0.46, 0.86, 1.8, 3.6 and 7.3 mg as/L.
4.2.2	Actual concentrations of test substance	
4.2.3	Effect data	See Tables A7.4.3.4/01-2 .3 and 4
4.2.4	Concentration / response curve	See Table A7.4.3.4/01-2 for survival (mortality) data.
4.2.5	Other effects	none
4.3	**Results of controls**	See Tables A7.4.3.4/01-2 and 3
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The effect of imidacloprid technical on the reproduction of water fleas was determined in a laboratory study under semistatic test conditions. In a study conducted according to US- EPA-FIFRA 72-4 guideline, 6 first instars of *Daphnia magna* (< 24 h old) per test chamber (4 replicates) were exposed under static renewal conditions for 21 d to nominal concentrations of 0 (control), 0.49, 0.94, 1.9, 3.8 and 7.5 mg a.s./L. Mean measured concentrations were 0.46, 0.86, 1.8, 3.6 and 7.3 mg as/L. Percent survival and adult length, young/adult reproduction and time to first brood were measured.
5.2	**Results and discussion**	Daphnid reproduction and percent survival were significantly affected at 7.3 mg as/L. The day 21 EC50 was estimated greater than 7.3 mg as/L. Adult daphnid length was significantly affected at 3.6 and 7.3 mg as/L. In the 21 d chronic test, the NOEC value was determined to be 1.8 mg as/L.
5.2.1	NOEC	1.8 mg a.s./L
5.2.2	LOEC	3.6 mg a.s./L based on adult Daphnid length
5.2.3	EC_{50} (EC_x)	>7.3 mg a.s./L
5.3	**Conclusion**	In a 21 days static renewal toxicity study with *Daphnia magna* meeting the validity criteria, the NOEC of imidacloprid (NTN 33893) was determined to be 1.8 mg as/L (mean measured concentration).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 7.4.3.4/01
Annex Point IIIA XIII 2.4

Effects on reproduction and growth rate with an invertebrate species

Chronic static renewal toxicity to Daphnia magna

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/13
Materials and Methods	Applicant's version is acceptable with the following comments/additions: 3.4: DMF was used as solvent (0.1 ml/l); the test design was semi-static with 3 renewals per week; photoperiod: 16:8 hours with 30 min dawn and dusk transition period; Daphnids were fed during the study twice per day with algae, trout chow and yeast. Temperature, oxygen content and pH were measured at each renewal in the fresh test solution and 1 per wek in composites of alternating replicates of the old solutions. Temperature: 19-22 °C (fresh solution); 20-21 °C (old solution) Oxygen content: 8.1 – 9.1 mg7l (fresh solution); 5.8 – 7.8 mg/l (old solution) pH: 8.1 – 8.5 (fresh solution); 7.9 – 8.5 (old solution)
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.4.3.4/01-1: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	ABC Laboratories in house culture, originally obtained from Columbia National Fisheries Research Laboratory
Age	<24 hours at study initiaition
Breeding method	Cultured in hard blended water (160-180 mg/L as CaCO3)
Kind of food	Adult daphnids fed on alga and trout chow and yeast
Amount of food	Not detailed, according to guideline
Feeding frequency	Twice daily
Pretreatment	Non, study initiated when daphnids were distributed to test vessels
Feeding of animals during test	Yes

Table A7.4.3.4/01-2 Percent survival and adult length of Daphnia magna continuously exposed to imidacloprid for 21 days

Test-Substance Concentration measured [mg/l]	Totals of 4 Replicates			
	Initial no. instars	Adults survival	Mean % survival ± S.D.	Day 21 Adult Mean length ± S.D
Control	24	24	100 ± 0.0	4.5 ± 0.10
Solvent control	24	23	96 ± 8.3	4.5 ± 0.10
0.46	23	22	95 ± 10.0	4.5 ± 0.11
0.86	24	24	100 ± 0.0	4.5 ± 0.07
1.8	24	24	100 ± 0.0	4.5 ± 0.07
3.6	24	23	96 ± 8.3	4.3 ± 0.08*
7.3	24	20	83 ± 13.6*	3.6 ± 0.15*

*denotes significantly different ($p \leq 0.05$) from the pooled controls

Table A7.4.3.4/01-3 Reproduction days and time to first brood of Daphnia magna continuously exposed to imidacloprid for 21 days

Test-Substance Concentration measured [mg/l]	Total of 4 replicates			
	Total young	Adult reprod. days	Mean Mean ± S.D. Young/Adult reprod. days	Time to first brood ± S.D
Control	5623	90, 90, 90, 90	15.7 ± 0.17	7.0 ± 0.0
Solvent control	5413	90, 90, 89, 90	15.1 ± 0.75	7.0 ± 0.0
0.46	5611	74, 90, 90,90	16.4 ± 0.68	7.0 ± 0.0
0.86	5758	90, 90, 90, 90	16.0 ± 0.41	7.0 ± 0.0
1.8	5915	90, 90, 90, 90	16.5 ± 0.35	7.0 ± 0.0
3.6	5313	90, 90, 89, 90	14.87 ± 0.49	7.0 ± 0.0
7.3	2535	90, 83, 76, 79	7.7 ± 0.65*	7.0 ± 0.0

*denotes significantly different ($p \leq 0.05$) from the pooled controls

Table A7.4.3.4/01-4: Imidacloprid: Reproduction of water fleas

Test substance	Tech. as
Test object	*Daphnia magna*
Exposure	21 d, semistatic
EC_{50} [mg as/L]	>7.3
Lowest observed effect concentration (LOEC) [mg as/L]	3.6*
No observed effect concentration (NOEC) [mg as/L]	1.8

* Based on adult daphnid length

Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	X	

Section 7.4.3.4/02 Annex Point IIIA XIII 2.4

Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2 7.2, II A, 8.2.7 /01*
	Authors (year)	Dorgerloh, M.; Sommer, H. (2001a)
	Title	Influence of imidacloprid (tech.) on development and emergence of larvae of *Chironomus riparius* in a water-sediment system
	Company, report No.	Bayer CropScience AG, Report-No.: DOM 21035 BES Ref. : M-075819-01-1
	Date	2001-10-04
	Testing facility	[redacted]
	Dates of work	February 22, 2001 – August 17, 2001
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR (Batch-No.: 230 924 394)
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**	BBA-proposal: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995); Proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (February 2001)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid (tech.), active substance-content: 98.4 % (Batch-No.: 230924394, Article-No.: 0004145852); Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none

Section 7.4.3.4/02 Annex Point IIIA XIII 2.4

Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius

3.1.6	Method of analysis	in water by HPLC MS-MS, Koenig, Method 00468, December 3, 1997, validation in report
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant, not poorly soluble
3.3	**Reference substance**	No
3.4	**Testing procedure**	
3.4.1	Dilution water	Study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR
3.4.2	Test organisms	Larvae of *Chironomus riparius* 1st instars < 2 - 3 days old (see Table A7.4.3.4/02-1)
3.4.3	Handling of offspring	See Tables A7.4.3.4/02-2 and 3. Study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR
3.4.4	Test system	
3.4.5	Test conditions	
3.4.6	Duration of the test	28 days
3.4.7	Test parameter	Water quality parameters (temperature, pH, oxygen) were measured weekly
3.4.8	Examination / Sampling	Test vessels were observed at least 3 times per week to make a visual assessment of behavioural differences. The sex, time and number of emerged or not fully emerged adults were recorded daily.
3.4.9	Monitoring of TS concentration	Yes, on days 0, 7 and 28 in test water and pore water for control and 0.35, 2.06 and 10 µg /L veseels
3.4.10	Statistics	Probit analysis for EC15 with regression analysis (Litchfield and Wilcoxon), χ2-test to establish emergence significance
		4 RESULTS
4.1	**Range finding test**	Reported as performed
4.1.1	Concentrations	1, 10 and 100 µg as/L
4.1.2	Number/ percentage of animals showing adverse effects	Not given in report
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0.35, 0.64, 1.14, 2.06, 3.70, 5.56 and 10.0 µg as/L (nominal) see Table A7.4.3.4/02-4 for analytical results of overlayer and pore water.

Section 7.4.3.4/02 Annex Point IIIA XIII 2.4

Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius

4.2.2	Actual concentrations of test substance		
4.2.3	Effect data	See Table A7.4.3.4/02-5 to 7. EC15 number of emerged midges at 2.25 µg as/L (C.I. limits 1.86-2.73 µg as/L) is the most sensitive end point. EC50= 3.11 µg as/L (C.I. limits 2.78-3.48 µg as/L)	
4.2.4	Concentration / response curve	slope of the line of regression was s=1.37	
4.2.5	Other effects	none	
4.3	**Results of controls**	See Tables A7.4.3.4/02-5 and 6	
4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of imidacloprid technical on *Chironomus riparius* over 28 days was determined in a laboratory water sediment system under static test conditions. In a study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, larvae of *Chironomus riparius* (1st instars < 2 - 3 days old, 3 beakers with 20 animals per test concentration and control) were exposed for 28 days in a static test system to concentrations of 0.35, 0.64, 1.14, 2.06, 3.70, 5.56 and 10.0 µg as/L (nominal) in a water-sediment system (spiked water). The sex, time and number of emerged or not fully emerged adults were recorded daily.	
5.2	**Results and discussion**	Within the concentration range of 0.35 to 1.14 µg as/L, high emergence ratios (81.7 – 83.8 %) were recorded. At test concentrations of 2.06 and 3.07 µg as/L the emergence was reduced to 68.3 % and 30 %, respectively. Start of emergence was postponed at 3.70 µg as/L. No emergence had been observed at the test levels of 5.56 and 10.0 µg as/L. The EC15 (probit analysis) for the development rate was not calculable, as the influence on the development rate was below 15 % at all test concentrations with emergence (0.35 – 3.70 µg as/L) compared to control findings. Thus, the emergence ratio (EC15 for the emergence ratio: 2.25 µg/L) was more sensitive than the development rate (EC15 of the development rate will be > 3.7 µg as/L and < 5.56 µg as/L).	x
5.2.1	NOEC	The corresponding EC15 as a surrogate for the NOEC was 0.002 mg as/L.	x
5.2.2	LOEC	-	
5.2.3	EC_{50} (EC_x)	0.003 mg a.s./L (95% C.I. 0.0028-0.0035 mg a.s./L)	
5.3	**Conclusion**	In a valid 28 days static toxicity test with *Chironomus riparius* the EC50 of imidacloprid for the endpoint emergence ratio was determined to be 0.003 mg as/L (nominal). The corresponding EC15 as a surrogate for the NOEC was 0.002 mg as/L.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section 7.4.3.4/02
Annex Point IIIA XIII 2.4

Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/13
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable with the following comment: 5.2: The given effect values are based on nominal concentrations. As the concentration of the test substance in the test system (overlying water and porewater) decreased during the exposure period, the use of nominal concentrations underestimates the toxicity of imidaclopride to Chironomus. 5.2.1: According to the TGD the surrogate for the NOEC is the EC10 (2.09 µg/l).
Conclusion	Applicant's version can be adopted considering the comments above.
Reliability	1
Acceptability	acceptable
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	

Table A7.4.3.4/02-1: Test organisms

Criteria	Details
Strain	*Chironomus riparius*
Source	University of Sheffield (UK) culture
Culture conditions	20 ± 2C, 16:8 h light:dark cycle
Age	Test animals of the first larval stage, parents of which stem from an approx. 21-28 day old synchronous culture
Breeding method	In cage, gauze on side, bottom of basin with layer of silica and 2-3 cm reconstituted water; 2-4 egg masses placed in prepared basin; hatched larvae are fed green alga and a vegetable fish food and after 2 to 3 weeks adults emerge; after mating females lay fresh egg masses on water surface; larvae used in study obtained by introducing fresh egg masses in small dishes with culture medium until hatch
Kind of food	green alga and a vegetable fish food
Amount of food	about 1 mg/larvae/day
Feeding frequency	Days -1,0,3,4,5,6,7,10,11,12,13,14,17,18,19,20,21,24, 25,26,27
Pre-treatment	None detailed
Feeding during test	On day 0 a suspension with commercial ornamental fish food was added to each test container

Table A7.4.3.4/02-2: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	600 ml, bottom covered with 1.5 cm sediment, 380 ml water
Volume/animal	Approx 20
Number of animals/vessel	20
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No
Test sediment	Artificial sediment, 74% fine quartz sand, 5% sphagnum peat, 20% kaolin, 1% CaCO3

Table A7.4.3.4/02-3: Test conditions

Criteria	Details
Test temperature	20 ± 2 C (20.1-20.4)
Dissolved oxygen	7.4-8.8 mg/l
pH	7.9-8.6
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	1081 lux
Photoperiod	16 hour daily
Total hardness day 0 and 28	0: Control 302.6 mg/l CaCO3, high dose vessel 284.8 mg/l CaCO3 28: 267.0 mg/l CaCO3 both vessels
Alkalinity	0: Control 195.8 mg/l CaCO3, high dose vessel 160.2 mg/l CaCO3 28: Control 89.0 mg/l CaCO3, high dose 71.2 mg/l CaCO3
Conductivity	568 µS/cm

Table A7.4.3.4/02-4 Analytical results in overlying water and pore water

Initial nominal conc. [µg a.s./l]	Mean of two analyses [µg/l]					
	1hour/day 0		Day 7		Day 28	
	Analyzed conc	% initial nominal	Analyzed conc	% initial nominal	Analyzed conc	% initial nominal
	Overlying water					
control	<0.029	-	n.a	-	n.a	-
0.35	0.33	94.3	0.12	34.3	0.072	20.6
2.06	1.93	93.7	0.75	36.4	0.46	22.3
10.0	9.53	95.3	4.82	48.2	2.50	25.0
	Pore water					
control	<0.029	-	n.a.	-	n.a.	-
0.35	<0.029	-	0.044	0.89	0.041	0.80
2.06	<0.3	-	0.31	1.06	<0.3	-
10.0	1.39	0.93	2.56	1.72	1.76	1.17

Table A7.4.3.4/02-5 Emerged midges over 28 days

Test-Substance Concentration nominal[µg/l]	Totals of 3 Replicates			
	No. Inserted larvae	No. Emerged midges	emergence % of inserted larvae	%male/%female emergence
Control	60	49	81.7	44.9/55.1
0.35	60	50	83.3	38.0/62.0
0.64	60	49	81.7	44.9/55.1
1.14	60	50	83.3	46.0/54.0
2.06	60	41	68.3	43.9/56.1
3.70	60	18	30.0	22.2/77.8
5.56	60	0	0	-
10.0	60	0	0	-

Table A7.4.3.4/02-6 Mean development time and rate of fully emerged midges (male and female pooled)

Test-Substance Concentration nominal[µg/l]	3 replicates		
	Total emerged*	Mean development time (d)	Mean development rate (1/d)
Control	49	15.40 ± 0.38	0.065 ± 0.002
0.35	50	15.98 ± 0.23	0.063 ± 0.001
0.64	49	15.73 ± 0.55	0.064 ± 0.002
1.14	50	15.41 ± 0.87	0.065 ± 0.004
2.06	41	15.14 ± 0.88	0.066 ± 0.004
3.70	18	17.59 ± 1.51	0.057 ± 0.005

*no midges emerged at the 2 highest concentrations

Table A7.4.3.4/02-7 Influence on the emergence and development after 28 days (based on nominal initial concentrations)

	EC_{15} (µg as/L)	95 % confidence limits (µg as/L)	EC_5 (µg as/L)	EC_{10} (µg as/L)	EC_{50} (µg as/L)
Emergence ratio (pooled sex)	2.25	1.86 – 2.73	1.86	2.09	3.11

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Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius for metabolite

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2 7.2, II A, 8.2.7 /02*
	Authors (year)	Dorgerloh, M.; Sommer, H. (2001b)
	Title	Influence of imidacloprid-desnitro on development and emergence of larvae of Chironomus riparius in a water-sediment system
	Company, report No.	Bayer CropScience AG, Report-No.: DOM 21039 BES Ref. : M-081499-01-1
	Date	2001-10-26
	Testing facility	[redacted]
	Dates of work	March 5, 2001- June 21, 2001
	Test substance(s)	Molecule(s): imidacloprid, WAK 4140 Substance(s): NTN 33893 Desnitro (Batch-No.: 960308ELB01)
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**	BBA-proposal: "Effects of plant protection products on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system" (1995); Proposal for a new OECD Guideline 219: "Sediment-Water Chironomid Toxicity Test Using Spiked Water" (February 2001)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	
3.1.1	Lot/Batch number	WAK 4140 NTN 33893 Desnitro (Batch-No.: 960308ELB01), purity 83.8%
3.1.2	Specification	
3.1.3	Purity	stability guaranteed for the duration of the study.
3.1.4	Composition of Product	Not relevant , not a b.p.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Desnitro in water by HPLC-UV, Appendix C in report

Section 7.4.3.4/03 Annex Point IIIA XIII 2.4 — Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius for metabolite

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not relevant, not poorly soluble
3.3	**Reference substance**	No
3.4	**Testing procedure**	
3.4.1	Dilution water	Study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR
3.4.2	Test organisms	Larvae of *Chironomus riparius* 1st instars < 2 - 3 days old (see Table A7.4.3.4/03-1)
3.4.3	Handling of offspring	See Tables A7.4.3.4/03-2 and 3. Study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, no water, handling of offspring, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR
3.4.4	Test system	
3.4.5	Test conditions	
3.4.6	Duration of the test	28 days
3.4.7	Test parameter	Water quality parameters (temperature, pH, oxygen) were measured weekly
3.4.8	Examination / Sampling	Test vessels were observed at least 3 times per week to make a visual assessment of behavioural differences. The sex, time and number of emerged or not fully emerged adults were recorded daily.
3.4.9	Monitoring of TS concentration	Yes, on days 0, 7 and 28 in test water and pore water for control and 4.0, 16 and 105 mg /L veseels
3.4.10	Statistics	Probit analysis for EC15 with regression analysis (Litchfield and Wilcoxon), χ2-test to establish emergence significance
		4 RESULTS
4.1	**Range finding test**	no
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	4, 8, 16, 32, 64 and 105 mg as/L (nominal) see Table A7.4.3.4/03-4 for analytical results of overlayer and pore water.
4.2.2	Actual concentrations of test substance	
4.2.3	Effect data	See Table A7.4.3.4/03-5 to 7.
4.2.4	Concentration / response curve	slope of the line of regression was s=1.86 for development, sum of male and female and 1.25 for number of emerged midges
4.2.5	Other effects	none
4.3	**Results of controls**	See Tables A7.4.3.4/03-5 and 6

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Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius for metabolite

4.4	**Test with reference substance**	Not performed	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of imidacloprid desnitro, the major metabolite found in water sediment studies, on *Chironomus riparius* over 28 days was determined in a laboratory water sediment system under static test conditions. In a study conducted in accordance with the 2001 proposal for a new OECD Guideline 219, larvae of *Chironomus riparius* (1st instars < 2 - 3 days old, 3 beakers with 20 animals per test concentration and control) were exposed for 28 days in a static test system to concentrations of 4, 8, 16, 32, 64 and 105 mg pure metabolite/L (nominal) in a water-sediment system (spiked water). The sex, time and number of emerged or not fully emerged adults were recorded daily.	
5.2	**Results and discussion**	For the test concentrations of 4 to 32 mg pure metabolite/L, high emergence ratios (88.3 – 93.3 %) were recorded. At 64 mg pure metabolite/L, the emergence ratio was significantly reduced (6.7 %), whereas emergence was totally suppressed at 105 mg/L. Start of emergence was postponed at 64 mg pure metabolite/L. Based on these findings, an EC15 (development rate, males) of 33.6 mg/L was calculated.	x
5.2.1	NOEC	The corresponding lowest EC15 as a surrogate for the NOEC was 33.61 mg as/L.	x
5.2.2	LOEC	-	
5.2.3	EC_{50} (EC_x)	EC50dev 79.17 mg pure metabolite/L (C.I. 72.94 – 87.69); EC50 emerg 45.99 mg/L (C.I. 41.93-50.44)	
5.3	**Conclusion**	In a valid 28 days static toxicity test with *Chironomus riparius* the lowest EC50 of imidaclopriddesnitro (M9) was determined to be 45.99 mg/L (nominal, pure metabolite) for the emergence ratio. The lowest EC15 as a surrogate for the NOEC was 33.61 mg/L for the development rate of males. The metabolite is over 1000 times less toxic to *Chironomus riparius* than parent compound.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

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Annex Point IIIA XIII 2.4

Effects on development with a sediment dwelling invertebrate species using spiked water

28 day toxicity to Chironomus riparius for metabolite

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/13
Materials and Methods	Applicant's version is acceptable
Results and discussion	Applicant's version is acceptable with the following comment: 5.2: The given effect values are based on nominal concentrations. As the concentration of the test substance in the test system (overlying water and porewater) decreased during the exposure period, the use of nominal concentrations underestimates the toxicity of the test substance to Chironomus. 5.2.1: According to the TGD the surrogate for the NOEC is the EC10 (27 mg/l).
Conclusion	Applicant's version can be adopted considering the comments above.
Reliability	1
Acceptability	acceptable
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	

Table A7.4.3.4/03-1: Test organisms

Criteria	Details
Strain	*Chironomus riparius*
Source	University of Sheffield (UK) culture
Culture conditions	20 ± 2C, 16:8 h light:dark cycle
Age	Test animals of the first larval stage, parents of which stem from an approx. 21-28 day old synchronous culture
Breeding method	In cage, gauze on side, bottom of basin with layer of silica and 2-3 cm reconstituted water; 2-4 egg masses placed in prepared basin; hatched larvae are fed green alga and a vegetable fish food and after 2 to 3 weeks adults emerge; after mating females lay fresh egg masses on water surface; larvae used in study obtained by introducing fresh egg masses in small dishes with culture medium until hatch
Kind of food	green alga and a vegetable fish food
Amount of food	about 1 mg/larvae/day
Feeding frequency	Days -1,0,3,4,5,6,7,10,11,12,13,14,17,18,19,20,21,24, 25,26,27
Pre-treatment	None detailed
Feeding during test	On day 0 a suspension with commercial ornamental fish food was added to each test container

Table A7.4.3.4/03-2: Test system

Criteria	Details
Renewal of test solution	No
Volume of test vessels	600 ml, bottom covered with 1.5 cm sediment, 380 ml water
Volume/animal	Approx 20
Number of animals/vessel	20
Number of vessels/ concentration	3
Test performed in closed vessels due to significant volatility of TS	No
Test sediment	Artificial sediment, 74% fine quartz sand, 5% sphagnum peat, 20% kaolin, 1% CaCO3

Table A7.4.3.4/03-3: Test conditions

Criteria	Details
Test temperature	20 ± 2 C (20.0-20.4)
Dissolved oxygen	7.0-8.8 mg/l
pH	7.9-8.6
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	1081 lux
Photoperiod	16 hour daily
Total hardness day 0 and 28	0: Control 338.2 mg/l CaCO3, high dose vessel 320.4 mg/l CaCO3 28: Control 356.0 mg/l CaCO3, high dose vessel 338.2 mg/l CaCO3
Alkalinity	0: Control 195.8 mg/l CaCO3, high dose vessel 160.2 mg/l CaCO3 28: Control 89.0 mg/l CaCO3, high dose 71.2 mg/l CaCO3
Conductivity	571 µS/cm

Table A7.4.3.4/03-4 Analytical results in overlying water and pore water

Initial nominal conc. [mg a.s./l]	Mean of two analyses [µg/l]					
	1hour/day 0		Day 7		Day 28	
	Analyzed conc	% initial nominal	Analyzed conc	% initial nominal	Analyzed conc	% initial nominal
	Overlying water					
control	<0.388	-	n.a	-	n.a	-
4	3.71	92.8	1.07	26.8	0.456	11.4
16	12.9	80.6	5.25	32.8	2.14	13.4
105	101	96.2	47.6	45.3	17.4	16.6
	Pore water					
control	<0.388	-	n.a.	-	n.a.	-
4	0.533	0.84	0.685	1.23	0.264	0.42
16	2.07	0.86	2.74	1.09	1.32	0.56
105	29.2	1.87	32.5	1.97	14.2	0.99

Table A7.4.3.4/03-5 Emerged midges over 28 days

Test-Substance Concentration nominal[mg/l]	Totals of 3 Replicates			
	No. Inserted larvae	No. Emerged midges	emergence % of inserted larvae	%male/%female emergence
Control	60	58	96.7	44.8/55.2
4	60	56	93.3	35.7/64.3
8	60	56	93.3	48.2/51.8
16	60	53	88.3	45.3/54.7
32	60	55	91.7	50.9/49.1
64	60	4	6.7	50.0/50.0
105	60	0	0	-

Table A7.4.3.4/03-6 Mean development time and rate of fully emerged midges (male and female pooled)

Test-Substance Concentration nominal[mg/l]	3 replicates		
	Total emerged*	Mean development time (d)	Mean development rate (1/d)
Control	58	16.29 ± 0.64	0.061 ± 0.002
4	56	16.57 ± 0.88	0.060 ± 0.003
8	56	16.15 ± 0.24	0.062 ± 0.001
16	53	16.30 ± 1.09	0.062 ± 0.004
32	55	17.59 ± 0.55	0.057 ± 0.002
64	4	25.33 ± 2.57	0.040 ± 0.004

*no midges emerged at the highest concentration

Table A7.4.3.4/02-7 Influence on the emergence and development after 28 days (based on nominal initial concentrations)

	EC_{15} (mg pure metabolite/L)	95 % confidence limits (mg pure metabolite/L)	EC_{50} (mg pure metabolite/L)	EC_{5} (mg pure metabolite/L)	EC_{10} (mg pure metabolite /L)
Emergence ratio (pooled sex)	36.52	32.52 – 41.37	45.99	31.89	34.57
Development rate (pooled sex)	41.63	38.98 – 44.18	79.17	28.55	35.75
Development rate (male)	33.61	19.49 – 71.36	84.52	19.56	27.01
Development rate (female)	47.05	38.78 – 57.23	91.03	31.93	40.24

Section A7.5.1.1/01

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.8, II A, 8.5 /01*
	Authors (year)	Anderson, J. P. E. (1988)
	Title	Influence of NTN 33893 on the microbial mineralization of carbon in soils
	Company, report No.	Bayer CropScience AG, Report-No.: AJO/54088 BES Ref. : M-006978-01-2
	Date	1988-04-11
	Testing facility	[redacted]
	Dates of work	not specified
	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**	BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987
2.2	**GLP**	No
2.3	**Deviations**	Not specified

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity: 95.8 %, specification: (Prod. no.: 180587, Tox no. 2071-51).
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properites	none
3.1.6	Method of analysis	analysis in test substrate not necessary
3.2	**Reference substance**	No
3.3	**Testing procedure**	

Section A7.5.1.1/01

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

3.3.1	Soil sample / inoculum / test organism	Freshly collected samples of a loamy sand and a silt soil (see Table A7.5.1.1/01-1and 2 for soil properties) were sieved (2 mm), and treated with 0.27 and 2.7 mg NTN 33893 as/kg dry weight soil (representing a 1X 0.20 and 10X 2.00 kg a.s./ha soil application rate). Controls remained untreated. One set of soils was incubated without further amendment (C-source was soil organic matter) while a second set was mixed with powdered lucerne-grass-green meal (5000 mg/kg dry wt. soil). Samples of each treatment in incubation vessels (similar to detailed in Greaves et. al., 1978) were incubated at 20 + 2 °C and 40 – 50 % soil water capacity in the dark. After 7, 14, 21 and 28 days, the quantities of carbon dioxide released from the soils were determined.	x
3.3.2	Test system		x
3.3.3	Application of TS	As solid	
3.3.4	Test conditions	According to BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987, considered valid by the RMS of the December 2005 91/414 DAR.	
3.3.5	Test parameter	inhibition of microbial carbon transformation	
3.3.6	Analytical parameter	CO_2 measurement	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	7, 14, 21, 28 days	
3.3.9	Monitoring of TS concentration	No, the studies on carbon and nitrogen mineralisation had not to be designed to measure rates of recovery following treatment, because imidacloprid is not intended for the use in preparations for soil sterilisation.	
3.3.10	Controls	control without test substance, with and without amendment	
3.3.11	Statistics	t-test with 5% probability for significant difference	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.267 and 2.667 mg a.s./kg dry soil weight	
4.2.2	Actual concentrations of test substance	verification not required	
4.2.3	Concentration/ response curve	Table A7.5.1.1/01-3, no significant differences acompared to controls	
4.2.4	Effect data	See Table A7.5.1.1/01-3 and 4	
4.2.5	Other observed effects	None	

Section A7.5.1.1/01

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

4.3	**Results of controls**	See Table A7.5.1.1/01-3
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study done according to BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987, freshly collected samples of a loamy sand and a silt soil were sieved (2 mm), and treated with 0.27 and 2.7 mg NTN 33893 as/kg dry weight soil. Controls remained untreated. One set of soils was incubated without further amendment (C-source was soil organic matter) while a second set was mixed with powdered lucerne-grass-green meal (5000 mg/kg dry wt. soil). Samples of each treatment were incubated at 20 + 2 °C and 40 – 50 % soil water capacity in the dark. After 7, 14, 21 and 28 days, the quantities of carbon dioxide released from the soils were determined.	x
5.2	**Results and discussion**	In the 28 day tests, imidacloprid, applied at 0.27 and at 2.7 mg/kg dry wt. soil, had no negative influence on soil respiration or mineralisation of added carbon in a silt soil and a loamy sand.	
5.2.1	NOEC	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)	
5.2.2	EC_{10}	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)	
5.2.3	EC_{50}	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)	
5.3	**Conclusion**	When used as recommended, imidacloprid will have no negative influence on the microbial transformation of organic carbon in field soils.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.5.1.1/01

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/07/13
Materials and Methods	Applicant´s version is acceptable with the following amendment: 3.3.1/3.3.2/5.1: The soil samples were not freshly sampled but were stored after sampling at 20 ± 2 °C for at least 7 but no longer than 28 days subject to the regulations of BBA VI, 1-1 Influence on the Activity of the Soil Microflora. The application rates of 0.267 mg as/kg soil and 2.67 mg/kg soil correspond to a rate of 0.403 and 4 kg a.s. per hectare, respectively assuming a soil depth of 10 cm and a soil particle density of 1.5 g/mL
Results and discussion	4.2.3: Concentration/response curve: As only two concentrations of the test substance have been examined, it is not possible to create a concentration/response curve. 5.2.1 NOEC: 2.67 mg/kg dry weight. soil Based on an organic carbon content of 2%: NOEC = NOEC * $Fom_{soil\ (standard)}/Fom_{soil(exp)}$ = 2.67 * 0.02/0.0123 = 4.34 mg/kg dry wt. soil [TGD, Part II, formula (71)] 5.2.2 $EC_{10:}$ > 2.67 mg a.s./kg dry weight soil 5.2.2 $EC_{50:}$ > 2.67 mg a.s./kg dry weight soil
Conclusion	Regarding to the results of this study, imidacloprid will not induce significant effects on soil respiration up to 2.67 mg/kg dry weight. soil. The NOEC therefore is 2.67 mg/kg and 4.34 mg/kg based on an organic carbon content of 2% respectively.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*

Section A7.5.1.1/01

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

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

Table A7.5.1.1/01-1: Microbial sample

Criteria	Details
Nature	Loamy sand soil sample
pH	5.3
Organic carbon content [% dry weight]	0.84
Nitrogen content [% dry weight]	0.08
Initial microbial biomass	265 mg C/kg DS soil

Table A7.5.1.1/01-2: Microbial sample

Criteria	Details
Nature	Silt soil sample
pH	4.8
Organic carbon content [% dry weight]	1.23
Nitrogen content [% dry weight]	0.17
Initial microbial biomass	935 mg C/kg DS soil

Table A7.5.1.1/01-3: Production of CO2: Cumulative Values

	Mg Carbon Dioxide/100 g dry weight soil*					
Days after treatment	Without amendment			With amendment		
	Mg NTN 33893 (a.i.)/kg dry weight soil					
	0	0.267	2.667	0	0.267	2.667
Loamy sand						
7	19.2	23.9	22.2	254.5	267.0	261.3
14	32.3	36.4	35.7	313.8	332.9	322.0
21	42.7	46.3	46.3	345.8	366.5	355.3
28	52.5	57.3	56.6	371.6	392.4	380.6
%Control	100.0%	109.0%	107.7%	100.0%	105.6%	102.4%
Silt						
7	20.3	22.0	26.3	226.6	232.6	229.6
14	38.4	40.2	45.6	295.7	305.4	300.5
21	57.5	59.3	66.1	337.4	347.6	343.4
28	74.4	74.7	82.2	367.6	377.1	369.9
%Control	100.0%	100.4%	110.5%	100.0%	102.6%	100.6%

*average from 4 soil samples

Table A7.5.1.1/01-4: Effects of imidacloprid tech. on non-target soil micro-organisms

Test substance	**Tech. as**	
Test object	**Soil micro-organisms; C-cycle (2 soils: silt, loamy sand)**	
Exposure	**28 d**	
mg as/kg dry weight soil	**0.27**	**2.7**
Corresponding to an application rate of	0.2 kg as/ha	2 kg as/ha
Result	no significant influence	no significant influence

Section A7.5.1.1/02

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.8, II A, 8.6 /10*
	Authors (year)	Anderson, J .P. E. (1999e)
	Title	Influence of imidacloprid (tech.) in mineralization of (carboxyl-14C) sodium acetate to 14CO2 in a slurry of soil and water
	Company, report No.	Bayer CropScience AG, Report-No.: AJO/196699 BES Ref. : M-048331-01-1
	Date	1999-07-29
	Testing facility	[redacted]
.	Dates of work	May 04, 1999 to May 20, 1999
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR (Batch-No.: 230 824 088)
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**	Following Principles of Good Laboratory Practice [Chemicals Law (Chem G) of 25 July, 1994, Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 C(97) 186/Final]
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	Not specified

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity: 98.6 %, batch no. 0414585/230824088
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	analysis in test substrate not necessary
3.2	**Reference substance**	No
3.3	**Testing procedure**	

Section A7.5.1.1/02

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

3.3.1	Soil sample / inoculum / test organism	Soil slurries containing 10 g dry wt silty sand (see Table A7.5.1.1/02-1) and 20 mL H2O were used to determine the influence of 0, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 mg imidacloprid as/kg dry wt. soil on the microbial mineralization of 2 µg [carboxyl-14C] sodium acetate to $^{14}CO2$. Imidacloprid was added as an acetonitrile solution to 100 ml screw cap flasks, acetonitrile allowed to evaporate, 20 ml deionized water and then 10 g dry weight of moist soil were added followed by [carboxyl-^{14}C] sodium acetate aqueous solution. Slurries were shaken in the dark at 175 rpm and 20 + 2 °C; the quantities of $^{14}CO2$ released were determined by liquid scintillation counting after 6, 24 and 48 hours.
3.3.2	Test system	
3.3.3	Application of TS	
3.3.4	Test conditions	
3.3.5	Test parameter	inhibition of microbial carbon transformation
3.3.6	Analytical parameter	$^{14}CO_2$ measurement
3.3.7	Duration of the test	48 hours
3.3.8	Sampling	6, 24 and 48 hours
3.3.9	Monitoring of TS concentration	No
3.3.10	Controls	control without test substance
3.3.11	Statistics	Not specified; instead, a ≥ 15% difference at end study is considered meaningful according to the most stringent of the guidelines
		4 RESULTS
4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	0, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 mg imidacloprid as/kg dry wt
4.2.2	Actual concentrations of test substance	Not required
4.2.3	Concentration/response curve	See Table A7.5.1.1/02-2; no treatment related effect noted
4.2.4	Effect data	See Table A7.5.1.1/02-2
4.2.5	Other effects	none
4.3	**Results of controls**	See Table A7.5.1.1/02-2
4.4	**Test with reference substance**	Not performed

Section A7.5.1.1/02

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a GLP study done according to Principles of Good Laboratory Practice [Chemicals Law (Chem G) of 25 July, 1994, Annex 1 and OECD Principles of Good Laboratory Practice (GLP) of November 26, 1997 C(97) 186/Final] and modeled after the experiments of Beelen et.al. (1991), soil slurries containing 10 g dry wt silty sand and 20 mL H2O were used to determine the influence of 0, 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 mg imidacloprid as/kg dry wt. soil on the microbial mineralization of 2 µg [carboxyl-^{14}C] sodium acetate to $^{14}CO_2$. Slurries were shaken in the dark at 175 rpm and 20 + 2 °C; the quantities of $^{14}CO_2$ released were determined by liquid scintillation counting after 6, 24 and 48 hours.
5.2	**Results and discussion**	During the 48 hour test, more than 60% of the applied dosage of ^{14}C-labelled actate was mineralized to $^{14}CO_2$ at all dose levels and control.
5.2.1	NOEC	> 10 mg a.s./kg dry weight soil
5.2.2	EC_{10}	> 10 mg a.s./kg dry weight soil (2 kg a.s./ha)
5.2.3	EC_{50}	> 10 mg a.s./kg dry weight soil (2 kg a.s./ha)
5.3	**Conclusion**	Data were evaluated following published guidelines for assessing the effects of plant protection products on mineralisation of substrates by soil micro-organisms. In the most stringent of these guidelines, differences between treated and control samples, which are = 15 at the end of an experiment, are evaluated as a meaningful effect. Using this 15 % criterion, imidacloprid did not have a meaningful effect on mineralisation. The NOEC for imidacloprid in these mineralisation tests is greater than 10 mg as/kg.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.5.1.1/02

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of carbon in soils

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2009/08/13
Materials and Methods	Applicant´s version is acceptable.
Results and discussion	Applicant´s version adopted.
Conclusion	Applicant´s version adopted.
Reliability	2 (but not applicable for biocidal evaluation)
Acceptability	Not acceptable for biocidal evaluation.
Remarks	The study is not applicable for biocidal evaluation but may be of scientific value for other questions. The study was not conducted according to a guideline for testing of microbial inhibition in soil. Thus, the study cannot be validated as standard microbial inhibition test in soil.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.5.1.1/02-1: Microbial sample

Criteria	Details
Nature	silty sand soil sample from Bayer CropScience AG farm Laacherhof, no plant protection products used on the field since 1981
pH	6.0
Organic carbon content [% dry weight]	0.7
Nitrogen content [% dry weight]	0.1

Table A7.5.1.1/02-2: Influence of imidacloprid (tech.) on microbial mineralisation of 14C-labelled sodium acetate to 14CO2 in slurries of silty sand soil (0.7 % C; pH 6.0) and water (a)

Imidacloprid (mg as/kg dry wt soil)	% of applied radioactivity mineralised to $^{14}CO_2$ (cumulative values)					
	6 h		24 h		48 h	
	% Mineralised (a)	Inhibition in % of control	% Mineralised (a)	Inhibition in % of control	% Mineralised (a)	Inhibition in % of control
0	38.0 ± 1.2	0	50.9 ± 3.0	0	64.3 ± 3.2	0
0.05	40.2 ± 0.5	0	50.0 ± 1.4	1.8	63.7 ± 2.0	0.9
0.1	39.8 ± 1.0	0	50.4 ± 2.0	1.0	64.5 ± 2.3	0
0.5	38.4 ± 4.8	0	51.9 ± 9.3	0	64.1 ± 11.9	0.3
1.0	38.0 ± 2.4	0	49.2 ± 4.1	3.3	64.0 ± 5.9	0
5.0	40.2 ± 0.5	0	52.7 ± 1.6	0	67.3 ± 2.0	0
10.0	36.8 ± 4.9	3.2	48.8 ± 7.3	4.1	61.9 ± 7.7	3.7

(a) Results are averages + standard deviation from 3 replicates.

Section A7.5.1.1/03

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of nitrogen in soils

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.8, II A, 8.5 /02*
	Authors (year)	Blumenstock, I. (1988)
	Title	Influence of NTN 33893 on the microbial mineralization of nitrogen in soils
	Company, report No.	Bayer CropScience AG, Report-No.: BSI/54288 BES Ref. : M-006964-01-2
	Date	1988-07-20
	Testing facility	[redacted]
	Dates of work	not specified
	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**	BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987
2.2	**GLP**	No
2.3	**Deviations**	Not specified

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity: 95.8 %, specification: (Prod. no.: 180587, Tox no. 2071-51).
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Analysis in test substrate not necessary
3.2	**Reference substance**	No

Section A7.5.1.1/03

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of nitrogen in soils

3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	Freshly collected samples of a loamy sand and a silt soil (see Table A7.5.1.1/01-1and 2 for soil properties) were sieved (2 mm), and treated with 0.27 and 2.7 mg NTN 33893 as/kg dry weight soil (representing a 1X 0.20 and 10X 2.00 kg a.s./ha soil application rate). Controls remained untreated. Soils then received no further treatment (N-source was soil organic matter) or were then amended with ammonium sulfate (1000 mg/kg dry wt. soil). Samples of each treatment in brown glass bottles were closed with air permeable plastic sheets and kept at 20 + 2 °C and 40 – 50 % soil water capacity in the dark. After 7, 14, 21 and 28 days, samples of the soil were extracted (1 N KCl) and the amounts of ammonium, nitrate and nitrite in the extracts were determined.	x
3.3.2	Test system		x
3.3.3	Application of TS	As solid	
3.3.4	Test conditions	BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987, considered valid by the RMS of the December 2005 91/414 DAR.	
3.3.5	Test parameter	inhibition of microbial mineralization of nitrogen	
3.3.6	Analytical parameter	Ammonium, nitrite and nitrite plus nitrate measurement	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	7, 14, 21, 28 days	
3.3.9	Monitoring of TS concentration	No, the studies on carbon and nitrogen mineralisation had not to be designed to measure rates of recovery following treatment, because imidacloprid is not intended for the use in preparations for soil sterilisation.	
3.3.10	Controls	control without test substance, with and without amendment	
3.3.11	Statistics	t-test with 5% probability for significance	
		4 RESULTS	
4.1	**Range finding test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.27 and 2.7 mg a.s./kg dry soil weight	
4.2.2	Actual concentrations of test substance	verification not required	
4.2.3	Concentration/ response curve	Table A7.5.1.1/03-3 and 4, no significant differences acompared to controls	
4.2.4	Effect data	See Table A7.5.1.1/03-3 to 5. No nitrite was found.	
4.2.5	Other effects	none	

Section A7.5.1.1/03

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of nitrogen in soils

4.3	**Results of controls**	See Table A7.5.1.1/03-3 and 4
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study done according to BBA VI, 1-1 Influence on the Activity of the Soil Microflora, March 1987, freshly collected samples of a loamy sand and a silt soil were sieved (2 mm), and treated with 0.27 and 2.7 mg NTN 33893 as/kg dry weight soil (representing a 1X 0.20 and 10X 2.00 kg a.s./ha soil application rate). Controls remained untreated. Soils then received no further treatment (N-source was soil organic matter) or were then amended with ammonium sulfate (1000 mg/kg dry wt. soil). Samples of each treatment in brown glass bottles were closed with air permeable plastic sheets and kept at 20 + 2 °C and 40 – 50 % soil water capacity in the dark. After 7, 14, 21 and 28 days, samples of the soil were extracted (1 N KCl) and the amounts of ammonium, nitrate and nitrite in the extracts were determined. x
5.2	**Results and discussion**	In the 28 day tests, imidacloprid, applied at 0.27 and at 2.7 mg/kg dry wt. soil, had no negative no negative influence on soil respiration or mineralisation of added nitrogen in a silt soil and a loamy sand.
5.2.1	NOEC	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)
5.2.2	EC_{10}	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)
5.2.3	EC_{50}	> 2.7 mg a.s./kg dry weight soil (2 kg a.s./ha)
5.3	**Conclusion**	When used as recommended imidacloprid will have no negative influence on the microbial transformation of nitrogen in field soils.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.5.1.1/03

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of nitrogen in soils

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2009/04/06
Materials and Methods	Applicant´s version is acceptable with the following amendments: 3.3.1/3.3.2/5.1: The soil samples were not freshly sampled but were stored after sampling at 20 ± 2 °C for at least 7 but no longer than 28 days subject to the regulations of BBA VI, 1-1 Influence on the Activity of the Soil Microflora. The application rates of 0.27 mg as/kg soil and 2.7 mg/kg soil correspond to a rate of 0.408 and 4 kg a.s. per hectare assuming a soil depth of 10 cm and a soil particle density of 1.5 g/mL
Results and discussion	Applicant´s version is acceptable with the following amendments: 4.2.3: Concentration/response curve: As only two concentrations (instead of five) of the test substance have been examined, it is not possible to create a concentration/response curve. 5.2.1 NOEC: 2.7 mg/kg dry weight. soil Based on an organic carbon content of 2%: NOEC = NOEC * $Fom_{soil\ (standard)}/Fom_{soil(exp)}$ = 2,7 * 0.02/0.0123 = 4.4 mg/kg dry wt. soil [TGD, Part II, formula (71)] 5.2.2 $EC_{10:}$ > 2.7 mg a.s./kg dry weight soil 5.2.3 $EC_{50:}$ > 2.7 mg a.s./kg dry weight soil
Conclusion	Applicant´s version adopted with the following amendments: Regarding to the results of this study, imidacloprid will not induce significant effects on mineralisation of added nitrogen in a silt soil and a loamy sand soil up to 2.7 mg/kg dry wt. soil. The NOEC therefore is 2.7 mg/kg and 4.4 mg/kg based on an organic carbon content of 2% respectively.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*

Section A7.5.1.1/03

Annex Point IIA7.4

Inhibition to microbial activity (terrestrial)

Influence on microbial mineralization of nitrogen in soils

Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.5.1.1/03-1: Microbial sample

Criteria	Details
Nature	Loamy sand soil sample
pH	5.3
Organic carbon content [% dry weight]	0.84
Nitrogen content [% dry weight]	0.08
Initial microbial biomass	265 mg C/kg DS soil

Table A7.5.1.1/03-2: Microbial sample

Criteria	Details
Nature	Silt soil sample
pH	4.8
Organic carbon content [% dry weight]	1.23
Nitrogen content [% dry weight]	0.17
Initial microbial biomass	935 mg C/kg DS soil

Table A7.5.1.1/03-3: Rate of mineralization of nitrogen in a loamy sand

Days after treatment	Mg nitrogen/week/k g dry weight soil*					
	Mg NTN 33893 (a.i.)/kg dry weight soil					
	0		0.27		2.7	
	Ammonium	Nitrate	Ammonium	Nitrate	Ammonium	Nitrate
Without amendment						
0- 7	-0.32	4.67	-0.71	5.37	-0.35	6.55
14-7	-0.36	3.91	-0.16	3.53	-0.27	3.69
21-14	0.29	1.35	0.22	2.25	0.47	1.69
28-21	-0.17	3.04	-0.39	1.93	-0.48	2.91
With amendment						
0- 7	-54.09	56.18	-55.79	55.30	-54.87	54.92
14-7	-68.92	68.80	-68.78	71.88	-69.69	72.08
21-14	-37.74	29.14	-38.80	28.90	-36.67	30.74
28-21	-35.12	14.22	-33.41	15.83	-36.34	15.07

*average from 3 soil samples

Table A7.5.1.1/03-4: Rate of mineralization of nitrogen in a silt

Days after treatment	Mg nitrogen/week/k g dry weight soil*					
	Mg NTN 33893 (a.i.)/kg dry weight soil					
	0		0.27		2.7	
	Ammonium	Nitrate	Ammonium	Nitrate	Ammonium	Nitrate
Without amendment						
0- 7	-0.30	7.75	0.14	7.60	-0.74	7.88
14-7	-0.18	7.23	-0.47	7.95	-0.19	6.91
21-14	-1.28	6.15	-1.17	5.81	-0.66	6.86
28-21	-0.12	6.14	-0.12	7.27	-0.47	6.42
With amendment						
0- 7	_13.17	9.69	-8.79	8.46	-7.86	9.18
14-7	0.86	15.17	-2.16	14.09	-0.88	16.16
21-14	-12.42	13.01	-19.09	14.67	-21.97	9.24
28-21	-17.32	16.42	-16.29	16.65	-13.40	17.91

*average from 3 soil samples

Table A7.5.1.1/03-5: Effects of imidacloprid tech. on non-target soil micro-organisms

Test substance	Tech. as	
Test object	soil micro-organisms; N-cycle (2 soils: silt, loamy sand)	
Exposure	28 d	
mg as/kg dry weight soil	0.27	2.7
Corresponding to an application rate of	0.2 kg/ha	2 kg/ha
Result	no significant influence	no significant influence

Section A7.5.1.2/01 Earthworm, acute toxicity test

Annex Point IIIA XIII 3.2

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.6.1, II A, 8.4.1 /01*
	Authors (year)	Heimbach, F. (1986b)
	Title	Acute toxicity of NTN 33893 (techn.) to earth worms
	Company, report No.	Bayer CropScience AG, Report-No.: HBF/RG 63 BES Ref. : M-006863-01-2
	Date	1986-11-10
	Testing facility	[redacted]
	Dates of work	October 17, 1986 to October 31, 1986
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 2/86)
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 207
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity: 92.8 %, Specification: (PT-No.: 2/86)
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Analysis in test substrate not necessary
3.2	**Reference substance**	Chloracetamide
3.2.1	Method of analysis for reference substance	Not given

Section A7.5.1.2/01 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

3.3	**Testing procedure**	
3.3.1	Preparation of the test substance	Not relevant
3.3.2	Application of the test substance	a.s. mixed with finely ground quartz and then added to test substratesoil
3.3.3	Test organisms	Table A7.5.1.2/01-1
3.3.4	Test system	Table A7.5.1.2/01-2, study conducted according to OECD 207, no deviations noted by the RMS in the December 2005 91/414 DAR.
3.3.5	Test conditions	
3.3.6	Test duration	14 day
3.3.7	Test parameter	Mortality and weight at end
3.3.8	Examination	7 and 14 days for mortality
3.3.9	Monitoring of test substance concentration	No, not required by the guideline
3.3.10	Statistics	Regression line after Litchfield and Wilcoxon
	4 RESULTS	
4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	0, 0.1, 1, 3.2, 10, 32, 100, 316, 1000 mg/kg artificial soil
4.2.2	Effect data (Mortality)	See Table A7.5.1.2/01-3 and 4. 10.7 mg/kg (8.7 – 13.3 mg/kg, 95% C.I., gradient of regression line s= 2.69)
4.2.3	Concentration / effect curve	100% mortality at dose levels of 32 mg/kg and higher
4.2.4	Other effects	None
4.3	**Results of controls**	
4.3.1	Mortality	See Table A7.5.1.2/01-3, no control mortality
4.3.2	Number/ percentage of earthworms showing adverse effects	
4.3.3	Nature of adverse effects	
4.4	**Test with reference substance**	Performed

Section A7.5.1.2/01 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

4.4.1	Concentrations	10, 18, 24, 32 and 56 mg/kg
4.4.2	Results	LC_{50} = 26.1 mg/kg
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an acute toxicity study on earthworms conducted according to OECD 207 guidelines, *Eisenia fetida* andrei (> 2 months old, with a mean body weight of 383 mg, 40 earthworms per test concentration) were exposed for 14 d in special test substrate containing nominal concentrations of 0.1, 1.0, 3.2, 10, 32, 100 and 1 000 mg as/kg artificial soil. The number of surviving animals and their weight alteration during the test were determined.
5.2	**Results and discussion**	The lowest concentration causing mortality was 10 mg as/kg dry weight of substrate. Below the test concentrations of imidacloprid causing mortality, weight changes in earthworms were only observed at 3.2 mg as/kg dry weight of substrate.
5.2.1	LC_0	$\geq$ 3.2 mg/kg
5.2.2	LC_{50}	10.7 mg/kg (8.7 – 13.3 mg/kg, 95% C.I., s= 2.69)
5.2.3	LC_{100}	$\leq$ 32 mg.kg
5.3	**Conclusion**	The LC_{50} value of imidacloprid to earthworms was determined to be 10.7 mg as/kg dry weight soil.
5.3.1	Other Conclusions	The NOEC value was determined to be 1.0 mg as/kg dry weight soil based upon weight effects observed at 3.2 mg/kg soil.
5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/13
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version is acceptable.
Reliability	1
Acceptability	acceptable
Remarks	-

Section A7.5.1.2/01 Earthworm, acute toxicity test
Annex Point IIIA XIII 3.2

	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	

Table A7.5.1.2/01-1: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida andrei*
Source of the initial stock	Prof. Graff, Forchungsanstalt fuu Landwirtschaft
Culturing techniques	22 + 2C, 70-90% RH, 12:12 h light/dark cycle, natural soil:peat:straw mixture (70:25:5)
Age/weight	383 mg, >2 months old
Pre-treatment	1 day prior to study, 20 + 2C, 70-90% RH, 12:12 h light/dark cycle, not fed

Table A7.5.1.2/01-2: Test system

Criteria	Details
Artificial soil test substrate	69% fine quartz, 20% kaolin, 10%, sphagnum peat pH6 $\pm$ 0.5, moisture content 25.7-33.2%
Test mixture	0, 0.1, 1, 3.2, 10, 32, 100, 316, 1000 mg a.s./kg artificial soil
Size, volume and material of test container	1.5 l preserving jars with glass lids
Amount of artificial soil (kg)/ container	0.5 kg (dry weight)
Nominal levels of test concentrations	0, 0.1, 1, 3.2, 10, 32, 100, 316, 1000 mg a.s. /kg soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	400 – 800 lux
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.1.2/01-3: Mortality data

Test Substance Concentration nominal [mg/kg artificial soil]	Mortality			
	Number		Percentage	
	7 d	14 d	7 d	14 d
0	0	0	0	0
0.1	0	0	0	0
1	0	0	0	0
3.2	0	0	0	0
10	8	0	80	55
32	39	40	97.5	100
100	40	40	100	100
316	40	40	100	100
1000	40	40	100	100
Temperature [°C]	20 ± 2C			
pH	6 ± 0.5			
Moisture content	70-90% RH			

Table A7.5.1.2/01-4: Acute toxicity of imidacloprid to earthworms

Test substance	Technical as
Test object	*Eisenia fetida*
Exposure	14 d
LC_{50} [mg as/kg d.wt].	10.7
Lowest observed effect concentration (LOEC) mg as/kg d.wt.	3.2
No observed effect concentration (NOEC) mg as/kg d.wt.	1.0

d.wt. dry weight soil

Table A7.5.1.2/01-5: Validity criteria for acute earthworm test according to OECD 207

	fulfilled	Not fulfilled
Mortality of control animals < 10%	X	

Section A7.5.2.1/01 Earthworm, reproduction study

Annex Point IIIA XIII 3.2

Eisenia fetida reproduction

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.6.2, II A, 8.4.2 /01*
	Authors (year)	Heimbach, F. (1999)
	Title	Influence of low concentrations of imidacloprid (tech.) on the reproduction of earthworms (*Eisenia fetida*)
	Company, report No.	Bayer CropScience AG, Report-No.: HBF/RG 301 BES Ref. : M-032798-01-1
	Date	1999-05-20
	Testing facility	[redacted]
	Dates of work	March 15, 1999 to May 11, 1999
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn,
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**	ISO/DIS 11268-2 (1996); BBA, Guidelines for the Testing of Plant Protection Products Within Registration, Part VI, 2 - 2, January 1994
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	5% peat instead of 10%

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid (tech.) , 98.6 %, specification: Batch-No.: 230824088, Article-No.: 04145852
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Analysis in test substrate not necessary
3.2	**Reference substance**	Carbendazim (36% formulated product)

Section A7.5.2.1/01 Earthworm, reproduction study

Annex Point IIIA XIII 3.2

Eisenia fetida reproduction

3.3	**Testing procedure**	
3.3.1	Application of the test substance	Mixed with soil
3.3.2	Test organisms	Table A7.5.2.1/01-1
3.3.3	Test system	Table A7.5.2.1/01-2. Study conducted according to ISO/DIS 11268-2 (1996); BBA, Guidelines for the Testing of Plant Protection Products Within Registration, Part VI, 2 - 2, January 1994, no signit=ficant deviations noted by the RMS of the December 2005 91/414 DAR.
3.3.4	Test conditions	
3.3.5	Test duration	8 weeks (4 weeks adults, 4 weeks juveniles)
3.3.6	Test parameter	Mortality and weight adults, number of surviving juveniles
3.3.7	Examination	4 weeks and 8 weeks
3.3.8	Monitoring of test substance concentration	No, not required by the guideline
3.3.9	Statistics	U-test of Wilcoxon, Mann and Whitney

4 RESULTS

4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	0, 56, 75, 100, 133, 178 µg/kg artificial soil
4.2.2	Effect data (Mortality)	See Table A7.5.2.1/01-3
4.2.3	Concentration / effect curve	No mortality was noted
4.2.4	Effect data (Reproduction)	See Table A7.5.2.1/01-4
4.2.5	Other effects	Weight, see Table A7.5.2.1/01-3
4.3	**Results of controls**	
4.3.1	Mortality	See Table A7.5.1.2/01-3, no adult control mortality
4.3.2	Number/ percentage of earthworms showing adverse effects	
4.3.3	Nature of adverse effects	

Section A7.5.2.1/01 Earthworm, reproduction study

Annex Point IIIA XIII 3.2

Eisenia fetida reproduction

4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	0.10, 0.25, 0.50 kg formulation/has
4.4.2	Results	NOEL = 0.25 kg/ha (0.032 kg a.s./ha), LOEL = 0.5 kg/ha (0.079 kg a.s./ha),
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an earthworm reproduction study conducted according to ISO/DIS 11268-2 (1996); BBA, Guidelines for the Testing of Plant Protection Products Within Registration, Part VI, 2 - 2, January 1994 guidelines, adult *Eisenia fetida* (4 x 10 animals per concentration) were exposed in an artificial soil to the concentrations of 56, 75, 100, 133, and 178 µg test substance/kg dry weight soil. After 4 weeks the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 4 weeks, the number of offspring was determined.
5.2	**Results and discussion**	No mortality or body weight reduction of adult earthworms was observed at any concentration. The number of offspring was not reduced at any concentration. Imidacloprid (tech.) did not effect the body weight and reproduction of earthworms at all test concentrations up to 178 µg/kg dry weight soil.
5.3	**Conclusion**	The NOEC for earthworms regarding sublethal effects is ≥ 0.178 mg as/kg dry weight soil. There was no mortality in the control higher than the limit for natural mortality (<10%) according to the test guidelines. Reproduction was greater than 114 juveniles per 10 adults, exceeding the acceptable reproductive output of 30 juveniles per 10 adults after 8 weeks.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/21
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	NOEC: > 0.178 mg a.s./kg dw
Reliability	1
Acceptability	acceptable
Remarks	-

Section A7.5.2.1/01 Earthworm, reproduction study

Annex Point IIIA XIII 3.2

Eisenia fetida reproduction

	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	

Table A7.5.2.1/01-1: Test organisms

Criteria	Details
Species/strain	*Eisenia foetida andrei*
Source of the initial stock	Prof. Graff, Forchungsanstalt fuu Landwirtschaft
Culturing techniques	22 + 2C, 70-90% RH, 12:12 h light/dark cycle, natural soil:peat:straw mixture (70:25:5)
Age/weight	400 mg adults
Pre-treatment	1 day prior to study, 20 + 2C, 70-90% RH, 16:8 h light/dark cycle

Table A7.5.2.1/01-2: Test system

Criteria	Details
Artificial soil test substrate	74% fine quartz, 20% kaolin, 5%, sphagnum peat, 1% finely ground cattle manure pH6 $\pm$ 0.5, moisture content 12.9-28.5%%
Test mixture	0, 56, 75, 100, 133, 178 µg a.s./kg artificial soil
Size, volume and material of test container	1.2 l plastic boxes covered with fine mesh gauze
Amount of artificial soil (kg)/ container	0.5 kg (dry weight)
Nominal levels of test concentrations	0, 56, 75, 100, 133, 178 µg a.s. /kg soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	400-800 lux
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.2.1/01-3: Mortality data

Test Substance Concentration nominal [µg/kg artificial soil]	Mortality			
	Number 28d	Percentage 14 d	Weight alteration of the survivors %	U-Test
0	0	0	+18 ± 3	-
56	0	0	+14 ± 4	-
75	0	0	+16 ± 7	-
100	0	0	+16 ± 2	-
133	0	0	+23 ± 2	+
178	0	0	+16 ± 2	-
Temperature [°C]	20 ± 2C			
pH	6 ± 0.5			
Moisture content	70-90% RH			

results of the U-test: - = weights of controls and treatment do not differ significantly (p=0.05)

+ = weights of controls and treatment do not differ significantly (p=0.05)

Table A7.5.2.1/01-4: Number of juvenile earthworms per surviving adult after 8 weeks of exposure

Test Substance Concentration nominal [µg /kg artificial soil]	Mortality			
	Numbers per adult number juveniles	variation coeff (%)	Juvenile worms %	U-Test
0	+12.2 ± 1.2	9.9	100	
56	+11.8 ± 1.6	14.0	97	-
75	+12.2 ± 2.4	19.5	100	-
100	+12.8 ± 1.8	14.2	105	-
133	+11.4 ± 1.8	15.9	94	-
178	+12.1 ± 2.5	21.0	99	-
Temperature [°C]	20 ± 2C			
pH	6 ± 0.5			
Moisture content	70-90% RH			

results of the U-test: - = weights of controls and treatment do not differ significantly (p=0.05)

+ = weights of controls and treatment do not differ significantly (p=0.05)

Table A7.5.2.1/01-5: Effects on earthworn reproduction after 8 weeks

Test substance	Imidacloprid (tech.)					
Test object	*Eisenia fetida*					
Exposure	8 weeks					
Concentrations (µg/kg dry weight soil)	**Control**	**56**	**75**	**100**	**133**	**178**
Mortality of adult earthworms (%) after 4 weeks	0	0	0	0	0	0
Weight increase of adult earthworms (%)	+18	+14	+16	+16	+23*)	+16
Number of offsprings per surviving adult	12	12	12	13	11	12

*) Significant difference to control findings (U-Test, p = 0.05)

Section A7.5.2.1/02 Collembola, reproduction study

Annex Point IIIA XIII 3.2

Inhibition of folsomia candida reproduction

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.7, II A, 8.6 /02*
	Authors (year)	Wilhelmy, H. (1999)
	Title	NTN 33893 - Inhibition of reproduction of collembola (*Folsomia candida*)
	Company, report No.	Bayer CropScience AG, Report-No.: ICR64081 BES Ref. : M-031094-01-1
	Date	1999-08-26
	Testing facility	[redacted]
	Dates of work	May 31, 1999 to June 28, 1999
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR (Batch-No.: 230 824 088)
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**	ISO/FDIS 11267 (1998)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	The number of instars per replicate in control should be > 100. In this study in four replicates of the control > 100 instars were observed. Only 1 replicate showed with 76 instars a lower reproduction rate. Then mean number of instars in control was 123 instars per replicate. Therefore the study is assumed to be valid.

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893 (purity: 98.6 % imidacloprid, specification: batch no. PT: 230824088, article no. 04145852)
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Analysis in test substrate not necessary
3.2	**Reference substance**	500 g/l parathion

Section A7.5.2.1/02 Collembola, reproduction study

Annex Point IIIA XIII 3.2

Inhibition of folsomia candida reproduction

3.3	**Testing procedure**	
3.3.1	Application of the test substance	Substance applied with an amount of deionized water to adjust artificial soil to about 50% of its maximum water holding capacity
3.3.2	Test organisms	Table A7.5.2.1/02-1. *Folsomia candida* (50 springtails per control, 60 springtails per test substance concentration)
3.3.3	Test system	Table A7.5.2.1/01-2 for system. Maintained at 20 ± 2C, light/dark cycle 16/8 h/day. Study conducted according to ISO/FDIS 11267 (1998), no deviations noted by the RMS of the December 2005 91/414 DAR.
3.3.4	Test conditions	
3.3.5	Test duration	28 days
3.3.6	Test parameter	Mortality for adults, number of surviving instars for reproduction
3.3.7	Examination	14 days (replicates reweighed and adjusted for moisture); number of parents and instars on day 28
3.3.8	Monitoring of test substance concentration	No
3.3.9	Statistics	T-test, ANOVA and Dunnett's test
		4 RESULTS
4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	0.3 , 0.6, 1.25, 2.5, 5.0 and 10 mg/kg test substance/kg dry weight soil
4.2.2	Effect data (Mortality)	See Table A7.5.2.1/02-3
4.2.3	Concentration / effect curve	graphs are found in the report for both reference substance and test substance
4.2.4	Effect data (Reproduction)	See Table A7.5.2.1/02-4
4.2.5	LC50/EC50, NOEC	See Table A7.5.2.1/02-5 LC50 mortality= 2.7 mg a.s. /kg soil (0.8-9.4, 95% C.I.) EC50 reproduction= 3.4 mg a.s./kg soil (1.3-8.6, 95% C.I.)
4.3	**Results of controls**	See Table A7.5.1.2/02-3 and 4
4.4	**Test with reference substance**	Performed once per testing season
4.4.1	Concentrations	0.05, 0.10, 0.20, 0.40, 0.80 mg/ kg dry weight
4.4.2	Results	LC50/EC50 adult mortality 0.17 mg/kg, reproduction 0.15 mg/kg NOEC adult mortality 0.1, reproduction 0.05 mg/kg dw

Section A7.5.2.1/02 Collembola, reproduction study

Annex Point IIIA XIII 3.2

Inhibition of folsomia candida reproduction

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a collembola reproduction study conducted according to ISO/FDIS 11267 (1998) guideline, adult *Folsomia candida* (6 x 10 animals per active substance concentration) were exposed in an artificial soil to the concentrations of of 0.3 , 0.6, 1.25, 2.5, 5.0 and 10 mg/kg test substance/kg dry weight soil. Test duration was 28 days. During exposure springtails were fed with granulated dry yeast. Mortality and reproduction were assessed.
5.2	**Results and discussion**	Parathion was used as toxic reference treatment (LC50 = 0.17 mg/kg for corrected adult mortality and 0.15 mg/kg for reproduction). Mean adult mortality after 28 days in the control group was 8%. Corrected adult mortality in a.s. treated artificial soil ranged from 0% (0.3 mg/kg) to 100% (10 mg/kg). The LOEC for adult mortality was 0.6 mg/kg. LC50 mortality= 2.7 mg a.s. /kg soil (0.8-9.4, 95% C.I.) The mean number of juveniles found in untreated control was 123. The mean number of juveniles of test treatments ranged from 153 (0.3 mg/kg) to 0 (10 mg/kg). The LOEC for reproduction was 2.5 mg/kg EC50 reproduction= 3.4 mg a.s./kg soil (1.3-8.6, 95% C.I.)
5.3	**Conclusion**	The NOEC for the reproduction of *Folsomia candida* exposed to imidacloprid amounts to 1.25 mg as/kg dry weight soil. The NOEC for mortality of adults is 0.3 mg as/kg soil dw. LC50 mortality= 2.7 mg a.s. /kg soil EC50 reproduction= 3.4 mg a.s./kg soil
5.3.1	Reliability	1
5.3.2	Deficiencies	None which affect the validity of the study

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	2006/11/21
Materials and Methods	Applicant's version is acceptable with the following comment: Table A7.5.2.1/02-2: The test substance concentration given in this table is wrong. It is: 0.3; 0.6; 1.25; 2.5; 5 and 10 mg/kg dw.
Results and discussion	Applicant's version is acceptable.
Conclusion	NOEC: 0.3 mg/kg dw (mortalioty) NOEC: 1.25 mg/kg dw (reproduction)
Reliability	1
Acceptability	acceptable
Remarks	-

Section A7.5.2.1/02 Collembola, reproduction study

Annex Point IIIA XIII 3.2

Inhibition of folsomia candida reproduction

	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	

Table A7.5.2.1/02-1: Test organisms

Criteria	Details
Species/strain	*Folsomia candida*
Source of the initial stock	Home bred at the test facility
Culturing techniques	20 + 2C in the dark, 40% moisture content, artificial soil 90%w/w calcium sulphate dehydrate and 10% w/w powdered activated charcoal
Age	10-12 day old
Pre-treatment	Egg clusters were transferred to freshly prepared breeding containers; after 48 hours egg clusters were removed and the hatched instars fed (dry yeast); overcrowding was avoidewd for the next 12 days to avoid growth impact. On day 12, springtails were sucked from breeding containers, counted, checked for damage, then transferred to test ocntainers

Table A7.5.2.1/02-2: Test system

Criteria	Details
Artificial soil test substrate	69% fine quartz, 20% kaolin, 10% peat, 0.4% calcium carbonate pH6 $\pm$ 0.5, moisture content approx. 25% maximal water capacity
Test mixture	0, 56, 75, 100, 133, 178 µg a.s. /kg soil
Size, volume and material of test container	glass beaker (volume 100 mL, Ø = 4.3 cm) covered with parafilm
Amount of artificial soil (kg)/ container	0.030 kg (wet weight)
Nominal levels of test concentrations	0, 56, 75, 100, 133, 178 µg a.s. /kg soil
Number of replicates/concentration	5 control, 6 per test substance rate
Number of springtails/test concentration	50 springtails per control, 60 springtails per test substance concentration
Number of springtails/container	10
Light source	400-800 lux
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.2.1/02-3: Adult mortality in % (0-28 days)

Conc.	Replicate No.						Mean	Corrected
mg/kg	1	2	3	4	5	6		
10	100	100	100	100	100	100	100(+)	100
5	80	90	60	70	70	60	72(+)	70
2.5	50	20	60	10	70	30	40(+)	35
1.25	20	50	70	50	50	10	42(+)	37
0.6	30	30	20	30	30	20	27(+)	21
0.3	0	0	20	20	10	0	8(-)	0
control	0	0	20	20	0	-	8	-

(+) significantly different to contro (t-test, p=0.05)

(-) no significant differences

Table A7.5.2.1/02-4: Number of juveniles after 28 days

Conc.	Replicate No.						Mean value	Coeff of variation %	Inhibition %
mg/kg	1	2	3	4	5	6			
10	3*	2*	3*	3*	1*	1*	0(+)	-	100
5	32	14	66	63	33	41	42(+)	48	66
2.5	89	85	55	68	74	96	78(+)	19	37
1.25	124	109	50	115	93	122	102(-)	27	17
0.6	131	130	128	107	167	101	127(-)	18	0
0.3	156	179	133	156	146	150	153(-)	10	0
control	168	74	109	150	114	0	123	30	-

* juveniles were found dead

(+) significantly different to contro (t-test, p=0.05)

(-) no significant differences

Table A7.5.2.1/02-5: Toxicity to *Folsomia candida*, laboratory test

Test substance	NTN 33893	
Test object	*Folsomia candida*	
Exposure	Artificial soil	
	Adult mortality	Reproduction
LOEC [mg/kg]	0.6	2.5
NOEC [mg/kg]	0.3	1.25

Section A7.5.2.1/03
Annex Point IIIA XIII 3.2

Predacious mite, extended laboratory toxicity study
Toxicity to Hypoaspis aculeifer

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.7, II A, 8.6 /01*
	Authors (year)	Bakker, F. M. (1999)
	Title	An extended laboratory dose-response study to evaluate the effects of imidacloprid tech. on the predaceous mite *Hypoaspis aculeifer* Canestrini (Acari: Gamasidae)
	Company, report No.	Bayer CropScience AG, Report-No.: B019HAE BES Ref. : M-041284-01-1
	Date	1999-04-01
	Testing facility	[redacted]
.	Dates of work	September 22, 1998 to November 2, 1998
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn,
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**	Barrett et al. (SETAC/ESCORT, 1994)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid (techn.), Batch -No. 230724310, article no. 04145852, purity: 98.3 %
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	Analysis in test substrate not necessary
3.2	**Reference substance**	P-Dimethoate and pyrazophos

Section A7.5.2.1/03 Predacious mite, extended laboratory toxicity study

Annex Point IIIA XIII 3.2

Toxicity to Hypoaspis aculeifer

3.3	**Testing procedure**	
3.3.1	Application of the test substance	Potter spray tower
3.3.2	Test organisms	See Tables A7.5.2.1/03-1 and 2.
3.3.3	Test system	Juvenile *Hypoaspis aculeifer* (20 protonymphs per test unit maintained in a climate chamber at 25-26 C and 60-65% RH) were exposed to a layer of LUFA 2.2 soil of about 3 mm high that was treated with the test products at rates of 6, 9, 16, 26, 43, 72, and 120 g as/ha in glass cages. Dependent on the availability of protonymphs, the test was performed in three separate runs. Exposure lasted for at least 14 to 23 days. Endpoints of the test were juvenile mortality after a 7-day exposure period, egg production of females following juvenile exposure to the two highest test rates during two consecutive oviposition periods, and the hatching success of these eggs.
3.3.4	Test conditions	
3.3.5	Test duration	
3.3.6	Test parameter	
3.3.7	Examinations	
3.3.8	Monitoring of test substance concentration	No
3.3.9	Statistics	Corrected mortality according to Abbott

4 RESULTS

4.1	**Filter paper test**	Not performed
4.2	**Soil test**	
4.2.1	Effect data (Mortality)	For the imidacloprid treatments, mortality after 7 days ranged from 0 % to 22 %, but was not related the test rate. The 22 % mortality was found at an intermediate rate (26 g/ha) and could be attributed to a single deviant test unit. Without this observation corrected mortality ranged from 0 % to 6 %. Imidacloprid was not shown to affect reproductive performance, neither regarding the egg production rate, nor the egg hatching success.
4.2.2	Effect data (Reproduction)	
4.2.3	Concentration / effect curve	Mortality was not related to dose rate,
4.2.4	LC50/EC50, NOEC	See Table A7.5.2.1/03-3
4.3	**Results of controls**	Water control, 13% mortality after 7 days, egg hatch success 93 $\pm$ 15%
4.4	**Test with reference substance**	Performed once per testing season
4.4.1	Concentrations	dimethoate @ 400 g/ha
4.4.2	Results	mortality: 100% with dimethoate at 7 days; pyrazophos was demonstrated NOT to be an acceptable toxic standard for *Hypoaspis*

Section A7.5.2.1/03 Predacious mite, extended laboratory toxicity study

Annex Point IIIA XIII 3.2

Toxicity to Hypoaspis aculeifer

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In an extended laboratory study conducted according to Barrett et al. (SETAC/ESCORT, 1994), juvenile *Hypoaspis aculeifer* (20 protonymphs per test unit) were exposed to a layer of LUFA 2.2 soil of about 3 mm high that was treated with the test products in glass cages. Dependent on the availability of protonymphs, the test was performed in three separate runs. Exposure lasted for at least 14 to 23 days. Afugan (as pyrazophos) and Danadim (as dimethoate) were used as toxic reference compounds.

Endpoints of the test were juvenile mortality after a 7-day exposure period, egg production of females following juvenile exposure to the two highest test rates during two consecutive oviposition periods, and the hatching success of these eggs.

5.2 Results and discussion

Dimethoate as toxic reference gave 100% mortality.

For the imidacloprid treatments, mortality after 7 days ranged from 0 % to 22 %, but was not related the test rate. The 22 % mortality was found at an intermediate rate (26 g/ha) and could be attributed to a single deviant test unit. Without this observation corrected mortality ranged from 0 % to 6 %. Imidacloprid was not shown to affect reproductive performance, neither regarding the egg production rate, nor the egg hatching success.

5.3 Conclusion

The NOEC for mortality and reproduction of the predatory mite *Hypoaspis aculeifer* exposed to imidacloprid (as techn.) exceeds the application rate of 120 g as/ha.

The results on predatory mite *Hypoaspis aculeifer* and the springtail *Folsomia candida* confirm the insecticidal rather than acaricidal activity of imidacloprid.

5.3.1 Reliability: 1

5.3.2 Deficiencies: None

Section A7.5.2.1/03 Predacious mite, extended laboratory toxicity study

Annex Point IIIA XIII 3.2

Toxicity to Hypoaspis aculeifer

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/07/09
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	NOEC:> 120 g/ha
Reliability	2
Acceptability	acceptable
Remarks	Reliability was changed from 1 to 2 as only 1 concentration was tested and no discrete effect value could be derived.
	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	

Table A7.5.2.1/03-1: Test organisms

Criteria	Details
Species/strain	*Hypoaspis aculeifer*
Source of the initial stock	University of Amsterdam, Dept. of Population biology and Systematics
Culturing techniques	Approx 50 females put in jars with a plaster bottom darkened with activated charcoal about 6 days before start of test; eggs laid in jars yield the protonymphs used in the study
Age	protonymphs
Pre-treatment	Transferred from culture jar to test unit at start of study

Table A7.5.2.1/03-2: Test system

Criteria	Details
Soil test substrate	LUFA 2.2 soil
Test mixture	Equiv to 6, 9, 16, 26, 43, 72, and 120 g as/ha
Test container	Glass cages (10x4x0.3cm) fitted with ventilation holes
Amount of artificial soil/container	3 mm depth
Nominal levels of test concentrations	Equiv to 6, 9, 16, 26, 43, 72 and 120 g as/ha
Number of replicates/concentration	1 in first run, 2 in second run
Number of *H.aculeifer*/test concentration	60
Number of *H.aculeifer s*/container	20
Light source	Units covered with dark paper in the environmental chamber
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7.5.2.1/03-3: Toxicity to *Hypoaspis aculeifer*, extended laboratory test

Test substance	**Imidacloprid, techn.**	
Test object	***Hypoaspis aculeifer***	
Exposure	**Sandy soil (LUFA 2.2)**	
	Juvenile mortality	**Reproduction**
NOEC [g as/ha]	> 120	> 120

Section 7.5.3.1.1/01 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with bobwhite quail

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.1.1, IIA 8.1.1/01*
	Authors (year)	[redacted] (1990a)
	Title	Technical NTN 33893: An acute oral LD50 with Bobwhite quail
	Company, report No.	Bayer CropScience AG, Report-No.: 100059 BES Ref. : M-006718-01-1
	Date	1990-03-01
	Testing facility	[redacted]
	Dates of work	August 10, 1989 – August 24, 1989
	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	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**	Yes, FIFRA 71-1
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN33893 techn., purity: 97.4 %, Specification (Batch No.: Pt7001/88), Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	a.s. delivered in capsule, no confirmatory analytical method required
3.2	**Administration of test substance**	oral administration of the test substance with gelatin capsules without carrier

Section 7.5.3.1.1/01 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with bobwhite quail

3.3	**Reference substance**	no
3.4	**Testing procedure**	
3.4.1	Test organisms	Imidacloprid was given as a single oral administration of the test substance with gelatin capsules without carrier to 10 (5 male and 5 female in separate cages) adult Bobwhite Quail (*Colinus virginianus*, 20 weeks-old, see Table A7.5.2.1.1/01-1) at doses of 25, 50, 100, 200, 400, and 800 mg as/kg bw.
3.4.2	Test system	
3.4.3	Diet	Agway Gamebird Ration, untreated
3.4.4	Test conditions	Per FIFRA 71-1, no deviations noted by the RMS of the December 2005 91/414 draft DAR
3.4.5	Duration of the test	14 days
3.4.6	Test parameter	Bodyweight and body weight gain, food consumption, mortality and symptoms
3.4.7	Examination / Observation	
3.4.8	Statistics	LC50 by probit analysis Bartlett's test of equal variance ANOVA for parametric procedures followed by William's test as appropriate Kruskal-Wallis test for non-parametric procedures followed by Dunn's summed rank test as appropriate

4 RESULTS

4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results Test Substance**	
4.2.1	Applied concentrations	25, 50, 100, 200, 400, and 800 mg as/kg bw.
4.2.2	Effect data (Mortality)	Deaths were noted in all exposure groups ≥ 50 mg as/kg bw. They were observed between 24 hours and 7 days after dosing (see Table A7.5.3.1.1/01-2).
4.2.3	Body weight	
4.2.4	Feed consumption	Clinical signs of toxicity noted in the intoxicated birds included fluffed feather coat, hyporeactivity, ataxia, immobility, and wing drop. A statistically significant decrease in body weight was noted on day 7 in all exposure groups ≥100 mg as/kg bw when compared to controls. The 800 mg as/kg bw dose group showed a statistically significant decrease in food consumption.
4.2.5	Concentration / response curve	LD50 152 mg/kg bw (103-227 95% CI); see Table A7.5.3.1.1/01-3.
4.2.6	Other effects	Postmortem examinations noted treatment related effects of emaciation and fluid filled crop, intestines, and colon at all concentrations where mortality occurred.

Section 7.5.3.1.1/01 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with bobwhite quail

4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted according to EPA FIFRA 163.71-1, 5 male and 5 female adult bobwhite quail received single oral doses of imidacloprid technical in gelatin capsules without carrier at dose levels of 25, 50, 100, 200, 400, and 800 mg as/kg bw. During the subsequent observation period of 14 days the birds were observed for mortality and symptoms.
5.2	**Results and discussion**	Deaths were noted in all exposure groups ≥50 mg as/kg bw. They were observed between 24 hours and 7 days after dosing. Clinical signs of toxicity noted in the intoxicated birds included fluffed feather coat, hyporeactivity, ataxia, immobility, and wing drop. A statistically significant decrease in body weight was noted on day 7 in all exposure groups ≥100 mg as/kg bw when compared to controls. The 800 mg as/kg bw dose group showed a statistically significant decrease in food consumption. Postmortem examinations noted treatment related effects of emaciation and fluid filled crop, intestines, and colon at all concentrations where mortality occurred.
5.2.1	LD_{50}	LD50 152 mg/kg bw (103-227 95% CI)
5.3	**Conclusion**	Based on this study, which meets the validity criteria, the LD50 value for bobwhite quail exposed to imidacloprid was determined to be 152 mg as/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 7.5.3.1.1/01 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with bobwhite quail

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/23
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.5.3.1.1/01-1: Test animals

Criteria	Details
Species/strain	*Colinus virginianus*
Source	Barrett's quail Farm, Houston, TX USA
Age (in weeks), sex and initial body weight (bw)	17 weeks when received, 20 weeks at test initiation, males and females, weight at start of study ranging from 202 to 244 g
Breeding population	All birds from same hatch, phenotypically similar to birds from wild populations, approaching first breeding
Amount of food	*Ad libitum*
Age at time of first dosing	20 weeks
Health condition / medication	healthy

Table A7.5.3.1.1/01-2: Mortality data for Bobwhite Quail

Test substance dosage level [mg/kg bw]	Mortalities and Time to Mortalities						
	Total mortalities per dose level				Percent mortality		
	Male	Female	Com-bined	Day post dosing (no. of birds)	Male	Female	Com-bined
Control 1 (0)	0/5	0/5	0/10	na	0	0	0
Control 2 (0)	0/5	0/5	0/10	na	0	0	0
25	0/5	0/5	0/10	na	0	0	0
50	1/5	0/5	1/10	4	20	0	10
100	1/5	3/5	4/10	1,2 (2),1	20	60	40
200	2/5	4/5	6/10	1,2,5(2),6,7	40	80	60
400	4/5	4/5	8/10	1(3),2(2),3(2),5	80	80	80
800	5/5	5/5	10/10	1(3),2,3(3),4(2),5	100	100	100
Temperature [°C] 18-22							
Relative humidity 45-70							

na=not applicable

Table A7.5.3.1.1/01 -3 : Acute oral toxicity to Bobwhite Quail

Test substance	Tech. as
Test object	Bobwhite quail (male, female)
LD_{50} [mg as/kg bw]	152
Lowest lethal effect dose (LLED) [mg as/kg bw]	50
Lowest observed effect dose (LOED) [mg as/kg bw]	50
No observed effect dose (NOED) [mg as/kg bw]	25

Table A7.5.3.1.1/03-4: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	

Section 7.5.3.1.1/02 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with Japanese quail

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: B.9.1.1, IIA 8.1.1/02*	
	Authors (year)	[redacted] (1988a)	
	Title	Acute oral LD 50 of NTN 33893 to japanese quail	
	Company, report No.	Bayer CropScience AG, Report-No.: VW-123 BES Ref. : M-006710-01-1	
	Date	1988-01-28	
	Testing facility	[redacted]	
	Dates of work	December 1, 1987 – December 15, 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	Companies with letter of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA § 71-1, BBA 1, 1 - 2 (June 1987), BBA Bull. 48, App. 1 (1979)	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	- test species (Japanese quail) is not provided for in EPA guideline 71-1 - 13 d post-treatment observation period instead of 14 d (deviates from EPA 71-1)	
		3 METHOD	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Imidacloprid, purity : 95.3 %, batch no.: 180587,	
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.	
3.1.3	Purity		
3.1.4	Composition of product	Not relevant for a.s.	
3.1.5	Further relevant properties	none	
3.1.6	Method of analysis	No analysis of prepared solutions done	x

Section 7.5.3.1.1/02 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with Japanese quail

3.2	**Administration of test substance**	intubation of test substance in 5 % gum arabic/water-solution as carrier with a flexible probe
3.3	**Reference substance**	no
3.4	**Testing procedure**	
3.4.1	Test organisms	Imidacloprid was administered as a single oral administration of the test substance (5 % gum arabic/water-solution as carrier) with a flexible probe into the goitre of 10 (5 male and 5 female) young Japanese quail (*Coturnix cot. japonica*, 9 to 12 weeks-old, Table A7.5.2.1.1/02-1) per dose level: 2.5, 5 , 10, 20, 40, and 80 mg as/kg bw nominal; analysed dosages were in the range of 95 to 110 % of nominal values (5 to 80 mg as/kg), except for the 2.5 mg as/kg dose group (25 % to 26 % higher figures in analysed samples). Hence, for the lowest dose group, analysed data will be considered. Dosing was followed by a subsequent observation period of 13 days.
3.4.2	Test system	
3.4.3	Diet	Kuekenstartefutter KST 60, untreated
3.4.4	Test conditions	Per EPA § 71-1, BBA 1, 1 - 2 (June 1987), BBA Bull. 48, App. 1 (1979), deviations noted by the RMS of the December 2005 91/414 draft DAR not related to test conditions
3.4.5	Duration of the test	13 days
3.4.6	Test parameter	Bodyweight and body weight gain, food consumption, mortality and symptoms, gross necropsy
3.4.7	Examination / Observation	
3.4.8	Statistics	LC50 by probit analysis, Litchfield and Wilcoxon
		4 RESULTS
4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results Test Substance**	
4.2.1	Applied concentrations	0, 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 mg as/kg bw.
4.2.2	Effect data (Mortality)	Mortalities related to the test substance occurred from a dose of 10 mg as/kg bw within the first 24 hours after administration of the test compound (see Table A7.5.3.1.1/02-2). One bird died in the 5 mg as/kg bw group on day 8 after fracture of the base of the skull.
4.2.3	Body weight	
4.2.4	Feed consumption	
4.2.5	Concentration / response curve	Toxic symptoms were observed in dose groups from 5 – 80 mg as/kg bw with slight apathy, tumbling and ptosis at 5 mg as/kg bw up to unconsciousness at dose levels 20 - 80 mg as/kg bw. In the post-treatment observation period, there were only temporary differences in weight gains and feed consumption of the treatment groups compared to the control group. The highest dose without observable toxic effects (NOEL) was determined to be 3.1 mg as/kg bw. LD50 31 mg/kg bw (22 to 50 95% CI, slope 2.40); see Table A7.5.3.1.1/01-3.

Section 7.5.3.1.1/02 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with Japanese quail

4.2.6	Other effects	The animals which have been sacrificed at the end of the experiment revealed that the genital organs of about half the animals had not yet been developed. This allows the conclusion that all animals were still immature at the time of the administration of the test substance. There were no treatment-related findings.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted according to EPA § 71-1, BBA 1, 1 - 2 (June 1987), BBA Bull. 48, App. 1 (1979), 5 male and 5 female 9-12 week old Japanese quail received single oral doses of imidacloprid technical (5% gum arabic/water solution as carrier) by intubation at target dose levels of 2.5, 5, 10, 20, 40, and 80 mg as/kg bw. During the subsequent observation period of 13 days the birds were observed for mortality, weight, feed consumption and toxic symptoms.
5.2	**Results and discussion**	Mortalities related to the test substance occurred from a dose of 10 mg as/kg bw within the first 24 hours after administration of the test compound. One bird died in the 5 mg as/kg bw group on day 8 after fracture of the base of the skull. Toxic symptoms were observed in dose groups from 5 – 80 mg as/kg bw with slight apathy, tumbling and ptosis at 5 mg as/kg bw up to unconsciousness at dose levels 20 - 80 mg as/kg bw. In the post-treatment observation period, there were only temporary differences in weight gains and feed consumption of the treatment groups compared to the control group. The highest dose without observable toxic effects (NOEL) was determined to be 3.1 mg as/kg bw. The LD50 value was determined to be 31 mg as/kg bw. The animals which have been sacrificed at the end of the experiment revealed that the genital organs of about half the animals had not yet been developed. This allows the conclusion that all animals were still immature at the time of the administration of the test substance. There were no treatment-related findings.
5.2.1	LD_{50}	LD50 31 mg/kg bw (22-50 95% CI)
5.3	**Conclusion**	Based on this study, which meets the validity criteria, the LD50 value for Japanese quail exposed to imidacloprid was determined to be 31 mg as/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 7.5.3.1.1/02 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with Japanese quail

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/23
Materials and Methods	Aplicant's version is acceptable with the following comment: 3.1.6: All test formulations were examined for their active ingredient content before and after completion of the administration (no information on method given).
Results and discussion	Aplicant's version is acceptable.
Conclusion	Aplicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.5.3.1.1/02-1: Test animals

Criteria	Details
Species/strain	*Coturnix cot. japonica*
Source	Graeflich Degenfeld Schomburg'sche Gutsverwaltung
Age (in weeks), sex and initial body weight (bw)	6 to 9 weeks when received, 2 or 3 week acclimation before dosing, male and female, weight at beginning of study from 106 to 172 g
Breeding population	All birds about the same age from the breeder, , approaching first breeding age
Amount of food	*Ad libitum*
Age at time of first dosing	Between 9 and 12 weeks
Health condition / medication	Healthy, received antibiotic in the diet prophylactically

Table A7.5.3.1.1/02-2: Mortality data for Japanese Quail

Test substance dosage level [mg/kg bw]	Mortalities and Time to Mortalities						
	Total mortalities per dose level				Percent mortality		
	Male	Female	Com-bined	Day post dosing (no. birds)	Male	Female	Com-bined
Control (0)	0/5	1/5	1/10	14	0	20	10
2.5	0/5	0/5	0/10	na	0	0	0
5	0/5	1/5	1/10*	8	0	20	10
10	1/5	0/5	1/10	1	20	0	10
20	0/5	1/5	1/10	2	0	20	10
40	3/5	4/5	7/10	m 1,2(2); f 2(4)	60	80	70
80	4/5	5/5	9/10	m 1, 2(3); f 2(5)	80	100	90
Temperature [°C] 17-21							
Relative humidity 25-51							

na=not applicable

*bird reported to have dies from injury (fracture of skull), not test substance

Table A7.5.3.1.1/02-3: Acute oral toxicity to Japanese Quail

Test substance	tech. as
Test object	Japanese quail (male, female)
LD_{50} [mg as/kg bw]	31
Lowest lethal effect dose (LLED) [mg as/kg bw]	10*
Lowest observed effect dose (LOED) [mg as/kg bw]	5.0
No observed effect dose (NOED) [mg as/kg bw]	3.1**

* Substance-related mortality
** Analysed concentration, explanation see above

Table A7.5.3.1.1/02-4:Validity criteria for avian acute oral toxicity test according toEPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	

Section 7.5.3.1.1/03 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with mallard ducks

Official use only

		1 REFERENCE
1.1	**Reference**	*PPP monograph: B.9.1.1, IIA 8.1.1/03*
	Authors (year)	[redacted] (1996)
	Title	NTN 33893 technical: An acute oral LD50 with mallards
	Company, report No.	Bayer CropScience AG, Report-No.: 107354 BES Ref. : M-006784-01-1
	Date	1996-06-20
	Testing facility	[redacted]
	Dates of work	January 23,1996 – February 6, 1996
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 17129/90, 1030010)
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**	Yes, FIFRA 71-1
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None
		3 METHOD
3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid techn., purity: 96.6 %, batch No.: 1030010/17129-90, Specification as given in section 2; stability guaranteed for the duration of the study
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	delivered in capsules, no confirmatory analysis required
3.2	**Administration of test substance**	oral administration of the test substance with gelatin capsules
3.3	**Reference substance**	no

Section 7.5.3.1.1/03 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with mallard ducks

3.4	**Testing procedure**	
3.4.1	Test organisms	Imidacloprid was given as a single oral administration of the test substance with gelatin capsules to 6 (3 male and 3 female in separate cages) adult mallards (*Anas platyrhynchos*, 19 weeks old, see Table A7.5.3.1.1/03-1) per dose level: 25, 50, 100, 200, 400, and 800 mg as/kg bw.
3.4.2	Test system	
3.4.3	Diet	Agway Gamebird Ration, untreated
3.4.4	Test conditions	Per FIFRA 71-1, no deviations noted by the RMS of the December 2005 91/414 draft DAR
3.4.5	Duration of the test	14 days
3.4.6	Test parameter	Bodyweight and body weight gain, food consumption, mortality and symptoms
3.4.7	Examination / Observation	
3.4.8	Statistics	LD50 probit Levene's test of equal variance, ANOVA, one-tailed Dunnett's test
		4 RESULTS
4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results Tesat Substance**	
4.2.1	Applied concentrations	0, 25, 50, 100, 200, 400, and 800 mg as/kg bw.
4.2.2	Effect data (Mortality)	See Table A7.5.3.1.1/03-2 for mortality data. The symptoms of intoxication (e.g., ataxia, hyporeactivity, immobility, and diarrhea) were noted at all dose levels, excluding controls. The severity and duration of symptoms apperared to increase with increasing dose. With the exception of the mortality on day 8, no toxic symptoms were noted past day seven post-dose. There was no compound-related effect on mean body weights for males or females. There was a statistically significant reduction in weight change for males during the first seven days in the 50 and 200 mg as/kg bw. dose levels compared to controls. Feed consumption was significantly less for females at dose levels ≥ 400 mg as/kg bw and for males at dose levels ≥ 200 mg as/kg bw. compared to controls. LD50 283 mg/kg bw (182-439 95% CI: slope = +6.630); see Table A7.5.3.1.1/03-3.
4.2.3	Body weight	
4.2.4	Feed consumption	
4.2.5	Concentration / response curve	
4.2.6	Other effects	Postmortem examinations revealed no compound related findings.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none

Section 7.5.3.1.1/03 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with mallard ducks

4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute oral toxicity study conducted according to EPA FIFRA § 71-1, 3 male and 3 female adult mallard ducks received single oral doses of imidacloprid technical in gelatin capsules at target dose levels of 0, 25, 50, 100, 200, 400, and 800 mg as/kg bw. During the subsequent observation period of 14 days the birds were observed for mortality and symptoms.
5.2	**Results and discussion**	Compound related mortalities occurred at dose levels ≥ 200 mg as/kg bw within 2 – 8 days after administration. The symptoms of intoxication (e.g., ataxia, hyporeactivity, immobility, and diarrhea) were noted at all dose levels, excluding controls. The severity and duration of symptoms apperared to increase with increasing dose. With the exception of the mortality on day 8, no toxic symptoms were noted past day seven post-dose. There was no compound-related effect on mean body weights for males or females. There was a statistically significant reduction in weight change for males during the first seven days in the 50 and 200 mg as/kg bw. dose levels compared to controls. Feed consumption was significantly less for females at dose levels ≥ 400 mg as/kg bw and for males at dose levels ≥ 200 mg as/kg bw. compared to controls. Postmortem examinations revealed no compound related findings.
5.2.1	LD_{50}	283 mg as/kg bw (182-439 95% CI)
5.3	**Conclusion**	Based on this study, which meets the validity criteria, the LD50 value for Mallard duck exposed to imidacloprid was determined to be 283 mg as/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section 7.5.3.1.1/03 Acute oral toxicity on birds

Annex Point IIIA XIII 1.1

Acute LD50 with mallard ducks

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/23
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.5.3.1.1/03-1: Test animals

Criteria	Details
Species/strain	*Anus playrhynchos*
Source	Whistling wings Inc. Hanover, IL USA
Age (in weeks), sex and initial body weight (bw)	16 weeks when received, 19 weeks at test initiation, males and females, weight at start of study ranging from approx 0.85-1.1 kg for females and 1.05-1.3 kg for males
Breeding population	All birds from same hatch, phenotypically similar to birds from wild populations, approaching first breeding
Amount of food	*Ad libium*
Age at time of first dosing	19 weeks
Health condition / medication	healthy

Table A7.5.3.1.1/03-2: Mortality data for Mallard Duck

Test substance dosage level [mg/kg bw]	Mortalities and Time to Mortalities						
	Total mortalities per dose level				Percent mortality		
	Male	Female	Combined	Day post dosing (no. of birds)	Male	Female	Combined
Control (0)	0/3	0/3	0/6	na	0	0	0
25	0/3	0/3	0/6	na	0	0	0
50	0/3	0/3	0/6	na	0	0	0
100	0/3	0/3	0/6	na	0	0	0
200	1/3	0/3	1/6	4	33	0	17
400	3/3	2/3	5/6	m 2,4(2); f 6,7	100	67	83
800	3/3	3/3	6/6	m 3(2),4; f 2,5,8	100	100	100
Temperature [°C] 21(70F)							
Relative humidity 51%							

na=not applicable

Table A7.5.3.1.1/03-3: Acute oral toxicity to Mallard duck

Test substance	Tech. as
Test object	Mallard ducks (male, female)
LD_{50} [mg as/kg bw]	283
Lowest lethal effect dose (LLED) [mg as/kg bw]	200
Lowest observed effect dose (LOED) [mg as/kg bw]	25
No observed effect dose (NOED) [mg as/kg bw]	< 25*

* Lowest dose tested

Table A7.5.3.1.1/03-4:Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	

Section 7.5.3.1.2/01 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 with mallard ducks

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.1.2, IIA 8.1.2/01*
	Authors (year)	[redacted] (1990b)
	Title	Technical NTN 33893: A subacute dietary LC50 with mallard ducks
	Company, report No.	Bayer CropScience AG, Report-No.: 100238 BES Ref. : M-006721-01-1
	Date	1990-08-22
	Testing facility	[redacted]
	Dates of work	October 26, 1989 – November 3, 1989
	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**	Yes, FIFRA 163.71-2b, ASTM Standard Practice (E857-81)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None specified

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, purity: 97.4 %, specification (Batch No.: Pt 17001/88), Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	a.s. in diet, HPLC with UV detection, reported with validation in study report

Section 7.5.3.1.2/01 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 with mallard ducks

3.2	**Administration of the test substance**	via diet containing nominal concentrations of 0, 78, 156, 312.5, 625, 1250, 2500 and 5000 mg as/kg feed (measured concentrations of 0, 69, 150, 270, 622, 1228, 2474 and 4797 mg as/kg feed)
3.3	**Reference substance**	no
3.4	**Testing procedure**	
3.4.1	Test organisms	Young (10 days old) Mallard ducks (*Anas platyrhynchs,* source [redacted]) groups of ten birds each were fed diets containing nominal concentrations of 0, 78, 156, 312.5, 625, 1250, 2500 and 5000 mg as/kg feed (measured concentrations of 0, 69, 150, 270, 622, 1228, 2474 and 4797 mg as/kg feed) for 5 days with a subsequent 3-day observation period on untreated feed (diet used: Teklad DU-11 Duck Starter).
3.4.2	Test system	
3.4.3	Diet	
3.4.4	Test conditions	Per FIFRA 163.71-2b, ASTM Standard Practice (E857-81), no deviations noted by the RMS of the December 2005 91/414 DAR
3.4.5	Duration of the test	8 days
3.4.6	Test parameter	Bodyweights and bodyweight gain, feed intake, clinical signs, mortality, gross necropsy
3.4.7	Examination / Observation	
3.4.8	Statistics	LC50 by appropriate method determined by computer program Control group means, t-test Bartlett's test of equal variance ANOVA for parametric procedures followed by Dunnett's Multiple Range test, Dunnett's test and William's test as appropriate

4 RESULTS

4.1	**Limit Test / Range finding test**	Rangefinding details not reported
4.2	**Results test substance**	
4.2.1	Applied concentrations	nominal concentrations of 0, 78, 156, 312.5, 625, 1250, 2500 and 5000 mg as/kg feed (measured concentrations of 0, 69, 150, 270, 622, 1228, 2474 and 4797 mg as/kg feed)
4.2.2	Effect data (Mortality)	No mortality could be observed up to the highest concentration tested. The subacute dietary LC50 of technical grade imidacloprid in Mallard ducks is > 4797 mg as/kg diet, corresponding to an LD50 of > 237 mg as/kg bw based on average feed consumption and body weight (4797 mg as/kg feed x 7.3 g feed/bird/day)/148 g bw) (see Table A7.5.3.1.2/01-2) .
4.2.3	Body weight	
4.2.4	Food consumption	
4.2.5	Concentration / response curve	Toxic symptoms (ataxia) occured only in the two top concentrations 2474 (one bird of ten) and 4797 (four birds of ten) mg as/kg diet.

Section 7.5.3.1.2/01 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 with mallard ducks

		A statistically significant decrease in body weight was noted on day 5 in all exposure groups ≥ 150 mg as/kg feed. However, the data showed that birds exposed to imidacloprid treated diets gained weight after they were returned to the imidacloprid-free feed, indicating recovery. Feed consumption data support the body weight effects. These data suggest unpalatability of treated feed.
4.2.6	Other effects	Gross pathology: Postmortem examinations revealed no treatment-related or compound-related lesions.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a subacute dietary toxicity study conducted according to EPA FIFRA § 71-2b, groups of 10 young (10 days old) Mallard ducks (*Anas platyrhynchos*) were fed diets containing nominal concentrations of 0, 78, 156, 312.5, 625, 1250, 2500 and 5000 mg as/kg feed (measuredn concentrations of 0, 69, 150, 270, 622, 1228, 2474 and 4797 mg as/kg feed) for 5 days with a subsequent 3-day observation period on untreated feed.
5.2	**Results and discussion**	No mortality could be observed up to the highest concentration tested. The subacute dietary LC50 of technical grade imidacloprid in Mallard ducks is > 4797 mg as/kg diet, corresponding to an LD50 of > 237 mg as/kg bw based on average feed consumption and body weight ((4797 mg as/kg feed x 7.3 g feed/bird/day)/148 g bw). Toxic symptoms (ataxia) occured only in the two top concentrations 2474 (one bird of ten) and 4797 (four birds of ten) mg as/kg diet. A statistically significant decrease in body weight was noted on day 5 in all exposure groups ≥ 150 mg as/kg feed. However, the data showed that birds exposed to imidacloprid treated diets gained weight after they were returned to the imidacloprid-free feed, indicating recovery. Feed consumption data support the body weight effects. These data suggest unpalatability of treated feed. Postmortem examinations revealed no treatment-related or compound-related lesions.
5.2.1	LC_0	≥4797 mg as/kg feed
5.2.2	LC_{50}	>4797 mg as/kg feed
5.2.3	LC_{100}	>4797 mg as/kg feed

Section 7.5.3.1.2/01 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 with mallard ducks

5.3	**Conclusion**	In a valid study, the LC50 for mallard ducks was calculated as >4797 mg as/kg feed corresponding to an LD50 of >237 mg as/kg bw.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/27
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.5.3.1.2/01-1: Test animals

Criteria	Details
Species/strain	*Anus platyrhynchos*
Source	Whistling Wings Inc. Hanover, IL USA
Age (in weeks), sex and initial body weight (bw)	1 day old when received, 10 days of age at test initiation, males and females (unsexed), weight at start of study ranging from 140 to 196 grams
Breeding population	All birds from same hatch, phenotypically similar to birds from wild populations, never bred
Amount of food	*Ad libitum*
Age at time of first dosing	10 days weeks
Health condition / medication	healthy

Table A7.5.3.1.2/01-2: Short term dietary toxicity of imidacloprid to Mallard ducks

Test substance	Techn. as
Test object	Mallard duck
Exposure	5 d dietary
LC_{50} [mg as/kg feed]	> 4797
Lowest lethal effect concentration (LLEC) [mg as/kg feed]	> 4797
Lowest observed effect concentration (LOEC) [mg as/kg feed]	150*
No observed effect concentration (NOEC) [mg as/kg feed]	69

* Based on food consumtion and body weight

Table A7.5.3.1.2/01-3: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	X	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	X	

Section 7.5.3.1.2/02 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 to japanese quail

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.1.2, IIA 8.1.2/02*
	Authors (year)	[redacted] (1996)
	Title	NTN 33893 techn. 5-day-dietary LC50 to japanese quail
	Company, report No.	Bayer CropScience AG, Report-No.: GMU / VW-177 BES Ref. : M-006792-02-1
	Date	1996-03-14, Amended: 2002-01-22
	Testing facility	[redacted]
	Dates of work	June 26, 1994 – July 4, 1994 (first test) July 10, 1994 – July 18, 1994 (second test)
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 816 255 037)
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 205, EPA 71-2, ASTM Standard Practice (E857-81)
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	Additional control groups

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, purity: 97.2 % (batch no.: 816255037), Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	HPLC with UV detection, for example see study A7.5.3.1/03

Section 7.5.3.1.2/02 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 to japanese quail

3.2	**Administration of the test substance**	Dietary concentrations of 313, 625, 1250, 2500 and 5000 mg as/kg feed in a first study and 20, 54, 144, 380, and 1000 mg as/kg feed in a second study
3.3	**Reference substance**	no
3.4	**Testing procedure**	
3.4.1	Test organisms	Young (14 days old) Japanese quails (*Coturnix coturnix japonica,* source [redacted], see Table A7.5.1.2/02-1) groups of ten birds were exposed for 5 days to dietary concentrations of 0, 313, 625, 1250, 2500 and 5000 mg as/kg feed in a first study and 0, 20, 54, 144, 380, and 1000 mg as/kg feed in a second study with a subsequent 3-day observation period on untreated feed. Diet analysis confirmed in all doses 95.4 % - 109 % of the nominal as content. Therefore, the results are based on nominal concentrations. Diet: Altromin 0711 from Altromin GmbH
3.4.2	Test system	
3.4.3	Diet	
3.4.4	Test conditions	Per OECD 205, EPA 71-2, ASTM Standard Practice (E857-81), no deviations noted by the RMS of the December 2005 91/414 DAR
3.4.5	Duration of the test	8 days
3.4.6	Test parameter	Bodyweights and body weight gain, feed intake, clinical signs, mortality, gross necropsy
3.4.7	Examination / Observation	
3.3.8	Statistics	LC50 by probit analysis Body weight and gain comparison of multiple means; 95% Bonferroni

4 RESULTS

4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Applied concentrations	Dietary concentrations of 0, 313, 625, 1250, 2500 and 5000 mg as/kg feed in a first study and 0, 20, 54, 144, 380, and 1000
4.2.2	Effect data (Mortality)	Mortalities and partly severe symptoms of intoxication (narcosis, apathy, loss of equilibrium) occurred in animals exposed to ≥ 313 mg as/kg diet (see Table A7.5.3.1.2/02-2) . Animals exposed to 144 mg as/kg diet exhibited no signs of toxicity.
4.2.3	Body weight	
4.2.4	Food consumption	A dose-dependent reduction in feed intake during the 5-day exposure period was observed in all groups given imidacloprid at concentrations of ≥ 313 mg as/kg diet. This reduced feed intake resulted in a diminished body weight gain. This growth depression could not be compensated during the 3-day post-treatment observation period. Animals exposed to 144 mg as/kg diet transiently showed a slightly reduced body weight on
4.2.5	Concentration / response curve	

		study day 5, but returned to control values at study termination (day 8). See Table A7.5.3.1.2/02-3 According to the *Guidance Document on Risk Assessment for Birds and Mammals; SANCO/4145* (2002) a simple conversion of the diet-related endpoint of the 5-days-dietary test (LC50 = 392 mg as/kg diet) to a daily dietary dose (DDD), which is relevant for the risk assessment, is not appropriate if a strong food avoidance has been observed. In those cases it is recommended rather to use the NOELst as the relevant endpoint of the risk assessment in order to avoid an unreliable DDD. Therefore, the short-term risk assessment is conducted on the basis of the NOEL, derived from the short-term NOEC according to the specifications provided in Table Table A7.5.3.1.2/02-4.
4.2.6	Other effects	Gross pathology: Examinations of dead or sacrificed birds revealed some unspecific organ alterations, but without any dose-response pattern. Due to the time between death and examination, many birds could not be evaluated pathologically, and the alterations observed are most likely artifacts from post-mortem putrefaction.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a subacute dietary toxicity study conducted according to OECD 205, groups of 10 young (14 days old) Japanese quails (*Coturnix coturnix japonica*) were exposed for 5 days to dietary concentrations of 0, 313, 625, 1250, 2500 and 5000 mg as/kg feed in a first study and 0, 20, 54, 144, 380, and 1000 mg as/kg feed in a second study with a subsequent 3-day observation period on untreated feed. Diet analysis confirmed in all doses 95.4 % - 109 % of the nominal as content.
5.2	**Results and discussion**	Mortalities and partly severe symptoms of intoxication (narcosis, apathy, loss of equilibrium) occurred in animals exposed to ≥ 313 mg as/kg diet. Animals exposed to 144 mg as/kg diet exhibited no signs of toxicity. A dose-dependent reduction in feed intake during the 5-day exposure period was observed in all groups given imidacloprid at concentrations of ≥ 313 mg as/kg diet. This reduced feed intake resulted in a diminished body weight gain. This growth depression could not be compensated during the 3-day post-treatment observation period. Animals exposed to 144 mg as/kg diet transiently showed a slightly reduced body weight on study day 5, but returned to control values at study termination (day 8).

Section 7.5.3.1.2/02 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Subacute dietary LC50 to japanese quail

		Examinations of dead or sacrificed birds revealed some unspecific organ alterations, but without any dose-response pattern. Due to the time between death and examination, many birds could not be evaluated pathologically, and the alterations observed are most likely artifacts from post-mortem putrefaction.
5.2.1	LC_0	≥144 mg as/kg feed
5.2.2	LC_{50}	392 mg as/kg feed (328-477 95% CI)
5.2.3	LC_{100}	>625 and <1250 mg as/kg feed
5.3	**Conclusion**	In a short term dietary study meeting the validity criteria, the LC50 for Japanese quail was calculated as 392 mg as/kg feed
5.3.1	Reliability	1
5.3.2	Deficiencies	No

	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/27
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
Date	**COMMENTS FROM ...** *(specify)*
Materials and Methods	*Give date of comments submitted*
Results and discussion	*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*
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	*Discuss if deviating from view of rapporteur member state*

Table A7.5.3.1.12/02-1: Test animals

Criteria	Details
Species/strain	*Coturnix cot. japonica*
Source	Gefluegelzucht H&E Kueberich
Age (in weeks), sex and initial body weight (bw)	14 days at study initiation, unsexed, weight at beginning of study from 106 to 172 g
Breeding population	All birds about the same age from the breeder, unbred
Amount of food	*Ad libitum*
Age at time of first dosing	14 days
Health condition / medication	Healthy

Table A7.5.3.1.2/02-2: Mortality data for Japanese Quail

Nominal dietary concentration	Mortality/syomptoms and time to mortality/symptoms									
	Total number of birds dead/number of birds showing symptoms on study day number:									
	0	1	2	3	4	5	6	7	8	% Mortality
First Study										
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
313 ppm	0/0	0/4	0/10	0/10	0/10	1/0	1/0	1/0	1/0	10
625 ppm	0/0	0/10	1/10	4/9	5/6	8/5	9/1	9/0	9/0	90
1250 ppm	0/0	4/10	7/6	10/3	10/-	10/-	10/-	10/-	10/-	100
2500 ppm	0/0	4/10	8/6	9/2	10/1	10/-	10/-	10/-	10/-	100
5000 ppm	0/0	3/10	10/1	10/-	10/-	10/-	10/-	10/-	10/-	100
Second Study										
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
20 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
54 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
144 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
0380 ppm	0/0	0/10	1/10	1/9	4/9	5/6	5/5	6/1	6/0	60
1000 ppm	0/0	0/10	2/10	9/8	10/1	10/-	10/-	10/-	10/-	100
Temperature [°C]	24-28 C									

Table A7.5.3.1.2/02-3: Short-term dietary toxicity of imidacloprid to birds

Test substance	Tech. as
Test object	Japanese quail
Exposure	5 d dietary
LC_{50} [mg as/kg feed]	392
Lowest lethal effect concentration (LLEC) [mg as/kg feed]	313
Lowest observed effect concentration (LOEC) [mg as/kg feed]	313
No observed effect concentration (NOEC) [mg as/kg feed]	144
No observed effect dose (NOED) [mg as/kg bw/d]	28.5

Table A7.5.3.1.2/02-4: Calculation of the NOELst (daily dietary dose) in the Japanese quail subchronic study based on the NOECst (dietary concentration)

Species	$NOEC_{st}$ based on nominal* dietary concentration [mg as/kg diet]	Mean food consumption [g/bird/day]	Mean body mass of group animals [g/bird]	Daily amount of as ingested [mg as/bird]	$NOEL_{st}$ based on daily dietary dose (DDD) [mg as/kg bw/day]
Japanese quail	144	9.4	49.9	1.4	28.5

* At the 144 mg as/kg diet – level, 102 % of nominal have been analytically determined

Table A7.5.3.1.2/02-5: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	X	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	X	

Section 7.5.3.1.2/03 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Age-related subacute dietary toxicity to bobwhite quail

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.1.2, IIA 8.1.2/03*
	Authors (year)	[redacted] (1996)
	Title	Age-related five day dietary toxicity of Imidacloprid to bobwhite quail
	Company, report No.	Bayer CropScience AG, Report-No.: SXR/VB 057 BES Ref. : M-006782-01-1
	Date	1996-11-14
	Testing facility	[redacted]
	Dates of work	June 28, 1996 – July 18, 1996
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR (Batch-No.: 230 624 050)
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 205, EPA 71-2
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	None specified

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid, purity: 98.4 %, specification (Batch No.: 230 624 050, Development No.:0414 5852)
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	HPLC with UV detection, reported with validation in study appendix
3.2	**Administration of the test substance**	via nominal dietary concentrations (techn. as) of 0,156, 312, 625, 1250and 2500 mg as/kg feed for chicks and 0, 625, 1250, 2500 and 5000 mg as/kg feed for adult birds

Section 7.5.3.1.2/03 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Age-related subacute dietary toxicity to bobwhite quail

3.3	**Reference substance**	no
3.4	**Testing procedure**	
3.4.1	Test organisms	Bobwhite quail (*Colinus virginianus*, souirce [redacted], see Table A7.5.3.1.3/03-1) chicks (14-days-old) and adults (>20 weeks old) were exposed for 5 days to nominal dietary concentrations (techn. as) of 0, 156, 312, 625, 1250and 2500 mg as/kg feed for chicks (10 chicks per test concentration of unknown sex) and 0, 625, 1250, 2500 and 5000 mg as/kg feed for adult birds (5 male and 5 females per test concentration). Following the exposure period, birds were maintained on untreated diet for a postobservation period of 6 days (chicks) and 16 days (adult birds), respectively. Diet used: Altromin 0711 from Altromin GmbH.
3.4.2	Test system	
3.4.3	Diet	
3.4.4	Test conditions	Per OECD 205, EPA 71-2, no deviations noted by the RMS of the December 2005 91/414 draft DAR
3.4.5	Duration of the test	11 days for chicks, 21 days for adults
3.4.6	Test parameter	Bodyweights and body weight gain, feed intake, clinical signs, mortality, gross necropsy
3.4.7	Examination / Observation	
3.4.8	Statistics	LC50 by probit analysis Bartlett's test of equal variance ANOVA for parametric procedures followed by William's test as appropriate Kruskal-Wallis test for non-parametric procedures followed by Dunn's summed rank test as appropriate

4 RESULTS

4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Applied concentrations	via nominal dietary concentrations (techn. as) of 0,156, 312, 625, 1250and 2500 mg as/kg feed for chicks and 0, 625, 1250. 2500 and 5000 mg as/kg feed for adult birds

Section 7.5.3.1.2/03 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Age-related subacute dietary toxicity to bobwhite quail

4.2.2	Effect data (Mortality)	The subacute oral LC50 of imidacloprid to 14 day-old Bobwhite chick is estimated to be 2225 mg/kg feed (1414-8386 mg.kg 95% CI, slope of 2.5). The corresponding value for adult Bobwhites was above the highest test concentration of 5000 mg/kg feed (see Table A7.5.3.1.2/03-2)
4.2.3	Body weight	
4.2.4	Food consumption	
4.2.5	Concentration / response curve	Dead chicks were noted in the 312, 1250 and 2500 mg/kg feed treatment groups (see Table A7.5.3.1.2/03-3). Clinical signs of toxicity were noted in all chick groups of ≥312 mg/kg feed and included apathy, diarrhea, and discoordinated movements. No reliable assessment of potential treatment-related effects on body mass development of these chick groups can be made due to statistical differences at study initiation. The 156 mg/kg treatment level did not significantly affect body mass development of 14 day old chicks. Feed consumption was reduced in chick groups at treatment levels of ≥ 1250 mg/kg. Only one male quail died in the 1250 mg/kg treatment group with adult quails (Table A7.5.3.1.2/03-3). Clinical signs of toxicity noted in all treatment groups included apathy, diarrhea, and discoordinated movements. However, findings at the 625 mg/kg treatment level concerned only one male and are considered as incidental rather than treatment-related. No significant differences in body mass were noted between the control and the different treatment groups. Feed consumption during the exposure period was reduced in all treatment groups in a dose-dependent manner.
4.2.6	Other effects	Gross pathology: Examinations on chicks revealed a reduction of spleen size in all treatment groups ≥ 312 mg/kg feed. No treatment-related effects could be observed for adult Bobwhite quails in the pathological examinations. However, reduction in spleen size was observed in the two top treatment levels for male Bowhite quails.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none
4.4	**Test with reference substance**	Not performed

Section 7.5.3.1.2/03 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Age-related subacute dietary toxicity to bobwhite quail

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a repeat dose oral toxicity study conducted according to OECD 205, EPA 71-2 guidelines, groups of 10 bobwhite quail chicks of unknown sex and groups of 5 male and 5 female adult (>20 weeks) bobwhite quail were exposed to nominal dietary concentrations of imidacloprid technical, of 0, 156, 312, 625, 1250and 2500 mg as/kg feed for chicks and 0, 625, 1250, 2500 and 5000 mg as/kg feed for adult birds.
Following the exposure period, birds were maintained on untreated diet for a postobservation period of 6 days (chicks) and 16 days (adult birds), respectively.

5.2 Results and discussion

The subacute oral LC50 of imidacloprid to 14 day-old Bobwhite chick is estimated to be 2225 mg/kg feed. The corresponding value for adult Bobwhites was above the highest test concentration of 5000 mg/kg feed.

Dead chicks were noted in the 312, 1250 and 2500 mg/kg feed treatment groups. Clinical signs of toxicity were noted in all chick groups of ≥312 mg/kg feed and included apathy, diarrhea, and discoordinated movements. No reliable assessment of potential treatment-related effects on body mass development of these chick groups can be made due to statistical differences at study initiation. The 156 mg/kg treatment level did not significantly affect body mass development of 14 day old chicks. Feed consumption was reduced in chick groups at treatment levels of ≥ 1250 mg/kg.

Only one male quail died in the 1250 mg/kg treatment group with adult quails. Clinical signs of toxicity noted in all treatment groups included apathy, diarrhea, and discoordinated movements. However, findings at the 625 mg/kg treatment level concerned only one male and are considered as incidental rather than treatment-related. No significant differences in body mass were noted between the control and the different treatment groups. Feed consumption during the exposure period was reduced in all treatment groups in a dose-dependent manner.

Gross pathology examinations on chicks revealed a reduction of spleen size in all treatment groups ≥ 312 mg/kg feed. No treatment-related effects could be observed for adult Bobwhite

5.2.1 LC_0 Unclear, since in both chicks and adults mortalities occurred at a dose followed by no mortality at the next higher dose

5.2.2 LC_{50} 2225 mg as/kg feed in 14 day chicks ; >5000 mg as/kg feed adults

5.2.3 LC_{100} > 2500 mg as/kg feed for chicks ; >5000 mg as/kg feed adults

5.3 Conclusion

In a repeat dose study meeting validity criteria, the LC50 values for chicks and adult Bobwhite quail exposed to imidacloprid were calculated to be 2225 mg as/kg feed and >5000 mg as/kg feed, respectively.

5.3.1 Reliability 1

Section 7.5.3.1.2/03 Short-term toxicity on birds

Annex Point IIIA XIII 1.2

Age-related subacute dietary toxicity to bobwhite quail

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/27
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
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	

Table A7.5.3.1.2/03-1: Test animals

Criteria	Details
Species/strain	*Colinus virginianus*
Source	Barrett's quail Farm, Houston, TX USA for adults, chicks were obtained from purchased adults which served as controls in the reproduction study
Age (in weeks), sex and initial body weight (bw)	Adults > 20 weeks, males and females, weight at start of study ranging from 197 to 370 g, chick weights 24.9 to 40.7g
Breeding population	All birds from same hatch, phenotypically similar to birds from wild populations, adults approaching first breeding
Amount of food	*Ad libitum*
Age at time of first dosing	Adults >20 weeks, chicks 14 days
Health condition / medication	healthy

Table A7.5.3.1.2/03-2: Short term dietary toxicity of imidacloprid to Bobwhite quails

Test substance	**Techn. as**	
Test object	**chicks (14 days)**	**adult quails m, f * (>20 weeks)**
Exposure	5 d dietary	
LC_{50} [mg as/kg feed]	2225	>5000
Lowest lethal effect concentration (LLEC) [mg as/kg feed]	312	1250
Lowest observed effect concentration (LOEC) [mg as/kg feed]	312**	1250**
No observed effect concentration (NOEC) [mg as/kg feed]	156	625

* m,f: male, female,

** based on body weight

Table A7.5.3.1.2/03-3: Mortality data for Bobwhite Quail Chicks and Adults

Nominal dietary concentration	Mortality/symptoms and time to mortality/symptoms Total number of birds dead/number of birds showing symptoms on study day number:										
	0	1	2	3	4	5	6	7	8	9-21	% Mortality
Bobwhite Chicks											
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
156 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
312 ppm	0/0	0/10	0/10	0/10	0/10	0/10	0/1	0/1	1/1	0/0	10
625 ppm	0/0	0/10	0/10	0/10	0/10	0/10	0/0	0/0	0/0	0/0	0
1250 ppm	0/0	0/10	0/10	0/10	0/10	0/10	0/0	0/0	1/0	1/0	10
2500 ppm	0/0	0/10	0/10	1/10	3/9	6/5	6/3	6/3	7/2	7/0	70
Bobwhite Adults											
0 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0
625 ppm	0/0	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0
1250 ppm	0/0	0/10	0/10	0/10	0/10	0/10	0/1	0/1	0/1	1/1	10
2500 ppm	0/0	0/10	0/10	0/10	0/10	0/10	0/0	0/0	0/0	0/0	0
5000 ppm	0/5	0/10	0/10	0/10	0/10	0/10	0/10	0/9	0/8	0/8 to 0/5	0

Temperature [°C]	22-27 C
Relative Humidity	45-70%

Table A7.5.3.1.2/03-4: Validity criteria for short-term toxicity test according to OECD 205

	Fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	X	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	X	

Section 7.5.3.1.3/01 Effects on reproduction of birds

Annex Point IIIA XIII 1.3

One generation reproduction study with bobwhite quail

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.1.3, IIA 8.1.3/01*
	Authors (year)	[redacted] (1991)
	Title	Technical NTN 33893: A one generation reproduction study with bobwhite quail
	Company, report No.	Bayer CropScience AG, Report-No.: 101203 BES Ref. : M-006723-01-1
	Date	1991-02-25
	Testing facility	[redacted]
	Dates of work	May 23, 1990 – November 16, 1990
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 1717102/89)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EPA 71-4, OECD 206
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	One minor deviation concerning measurement of room temperature

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity: 94.8 %, Specification (Batch No.: 1717102-89); Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.2	Specification	
3.1.3	Purity	
3.1.4	Composition of Product	Not relevant for a.s.
3.1.5	Further relevant properties	none
3.1.6	Method of analysis	HPLC with UV detection, validation reported in study appendix

Section 7.5.3.1.3/01 Effects on reproduction of birds

Annex Point IIIA XIII 1.3

One generation reproduction study with bobwhite quail

3.2	**Administration of the test substance**	In diet, nominal (mean measured) dietary concentrations were 30 (36), 60 (61), 120 (126) and 240 (243) mg as/kg diet
3.3	**Testing procedure**	
3.3.1	Test organisms	See Table A7.5.3.1.3/01-1.
3.3.2	Test system	Imidacloprid was administered in the diet to groups of 18 pairs of young adult Northern Bobwhite quails per treatment (20 weeks old at test initiation); nominal (mean measured) dietary concentrations were 30 (36), 60 (61), 120 (126) and 240 (243) mg as/kg diet administered for 20 weeks.
3.3.3	Diet	Adult birds Agway Gamebird Ration, Wildlife International formula with test substance; offspring Teklad JQ-15 Quail Starter without test substance
3.3.4	Test conditions	Per US EPA 71-4, OECD 206, no significant deviations noted by the RMS of the December 2005 91/414 DAR
3.3.5	Duration of the test	Approx. 29 weeks in 5 phases: 18 day acclimation; 7 week photostimulation; 4 week pre-egg laying; 10 week egg laying; six week final incubation, hatching and 14 day offspring rearing
3.3.6	Test parameter	Feed consumption, body weight, clinical appearance, survival and postmortem findings of the parental generation were monitored;
3.3.7	Examination / Observation	effects on reproduction and first generation offspring were determined by measuring egg production, viability (fertility), 21-day embryo survival, hatchability and hatchling body weight, 14-day survival and survivor body weight and eggshell strength/thickness; a gross pathology was conducted.
3.3.8	Statistics	Bartlett's test of equal variance, ANOVA using F distribution for parametric procedures, Kruskal Wallis test for non-parametric procedures
		4 RESULTS
4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Applied concentrations	Nominal (mean measured) dietary concentrations were 30 (36), 60 (61), 120 (126) and 240 (243) mg as/kg diet

Section 7.5.3.1.3/01 Effects on reproduction of birds

Annex Point IIIA XIII 1.3

One generation reproduction study with bobwhite quail

4.2.2	Effect data (Mortality and reproductivity)	No treatment related mortalities occurred. See Table A7.5.3.1.3/01-2 for reproductive parameters. There was a significant reduction on the eggshell thickness, in view of the other reproduction parameters the effect was not considered biologically significant. Based on these results, the no observed effect concentration for the reproductive performance of Bobwhite quails exposed to imidacloprid techn. as in the diet was 126 mg as/kg food. The LOEC value was determined as 243 mg as/kg food. See A7.5.3.1.3/01-3. As the endpoints of the chronic bird studies (NOEC) were determined related to the concentrations in avian diet, the diet-related endpoints [mg as/kg diet] had to be converted to endpoints based on the daily dietary dose (NOEL) [mg as/kg bw/day], in order to assess the long-term risk in compliance with SANCO/4145 (2002). This was performed in detail as demonstrated in A7.5.3.1.3/01-4.
4.2.3	Body weight	Significant effects on body weight for females did not occur. However, mean body weight of males at 243 ppm was significantly reduced. There were significant reductions on hatchling body weight (with no clear dose-response trend). In view of the other reproduction parameters, the effect was not considered biologically significant.
4.2.4	Food consumption	
4.2.5	Results of residue analysis	Not performed
4.2.6	Other effects	Gross pathology: All adult birds were examined for gross lesions upon sacrifice or death. All observations were considered incidental and not related to the test material.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	none
4.3.2	Nature of adverse effects	none

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a one generation reproduction study conducted according to OECD 206 and EPA FIFRA § 71-4, imidacloprid was administered in the diet to groups of 18 pairs of young adult Northern Bobwhite quails per treatment (20 weeks old at test initiation); nominal (mean measured) dietary concentrations were 30 (36), 60 (61), 120 (126) and 240 (243) mg as/kg diet administered for 20 weeks; feed consumption, body weight, clinical appearance, survival and postmortem findings of the parental generation were monitored; effects on reproduction and first generation offspring were determined by measuring egg production, viability (fertility), 21-day embryo survival, hatchability and hatchling body weight, 14-day survival and survivor body weight and eggshell strength/thickness; a gross pathology was conducted.

Section 7.5.3.1.3/01 Effects on reproduction of birds

Annex Point IIIA XIII 1.3

One generation reproduction study with bobwhite quail

5.2	**Results and discussion**	There were no treatment-related mortalities, signs of toxicity or treatment related adverse effects on feed consumption of adult birds. Significant effects on body weight for females did not occur. However, mean body weight of males at 243 ppm was significantly reduced. There were significant reductions on hatchling body weight (with no clear dose-response trend) and on the eggshell thickness. In view of the other reproduction parameters, both effects were not considered biologically significant. Based on these results, the no observed effect concentration for the reproductive performance of Bobwhite quails exposed to imidacloprid techn. as in the diet was 126 mg as/kg food. The LOEC value was determined as 243 mg as/kg food. Gross pathology: All adult birds were examined for gross lesions upon sacrifice or death. All observations were considered incidental and not related to the test material.
5.2.1	NOEC	126 mg as/kg food
5.3	**Conclusion**	In a study meeting the validity criteria, the no observed effect concentration (NOEC) for Bobwhite quail exposed to imidacloprid techn. in the diet was 126 mg as/kg feed based on effects on body weight of male adults.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/27
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-

Section 7.5.3.1.3/01 Effects on reproduction of birds

Annex Point IIIA XIII 1.3

One generation reproduction study with bobwhite quail

	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	

Table A7.5.3.1.3/01-1: Test animals

Criteria	Details
Species/strain	*Colinus virginianus*
Source	Barrett's Quail Farm, Houston, TX USA
Age (in weeks), sex and initial body weight (bw)	Approx 20 weeks at test initiation, males and females, weight at atart of study approx 240g
Age range within the test	Approx 20 to 40 weeks (adult birds sacrificed at end of 20 week adult exposure period)
Breeding population	Approaching first breeding season when received
Amount of food	*Ad libitum*
Age at time of first dosing	Approx 20 weeks
Health condition / medication	healthy
Pre-treatment	2% mortality (5/266), dehydration and emaciation due to birds inability to adjust to caging