Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/04/05
Materials and Methods	Acceptable
Results and discussion	The applicant's version is acceptable.
Conclusion	The presented metabolism study in lactating goats has been previously described in the imidacloprid DAR for Annex I inclusion according to Directive 91/414/EEC. Crop use related MRLs for imidacloprid in EU Member States have been defined and the proposed residue definition for risk assessment is "imidacloprid including the degradation and reaction products, determined as 6-chloronicotinic acid; calculated as imidacloprid", the residue definition for monitoring is "sum of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, expressed as imidacloprid". Measurable residues in food or feed from the use of imidacloprid in PT18 biocidal products are not expected. Therefore, an additional exposure to humans through diet arising from the use of imidacloprid as a biocide can be excluded. No MRLs specific to biocidal product uses are necessary.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.15.3/01-1 Reference substances used for identification of metabolites

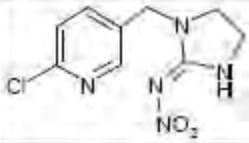
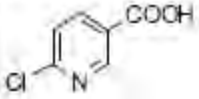
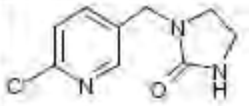
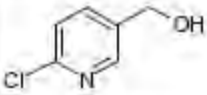
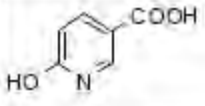
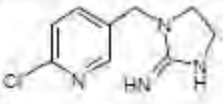
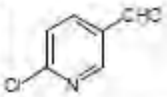
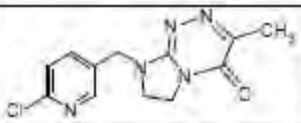
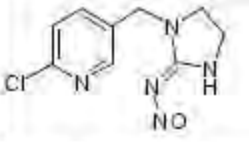
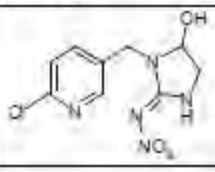
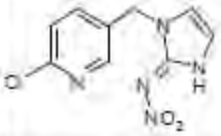
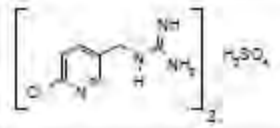
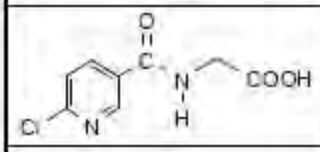
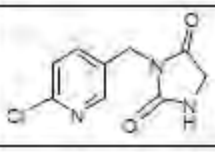
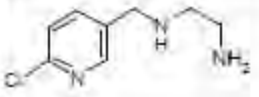
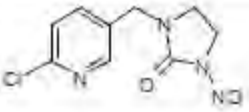
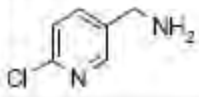
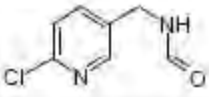
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid as		imidacloprid-6-CNA **M14**
	imidacloprid-urea **M12** (NTN33519)		imidacloprid-CHMP **M28** (DIJ9805)
	6-hydroxy-nicotinic acid (**M18**) (GBH4315)		imidacloprid-desnitro NTN38014 **M09**
	MAT 10249-0		imidacloprid-triazinone **M25**
	imidacloprid-nitrosimine **M07** (WAK3839)		imidacloprid-5-hydroxy **M01** (WAK4103)
	imidacloprid-olefine **M06** (NTN 35884)		imidacloprid-ring-open-guanidine **M10** (WAK4126)
	imidacloprid-6-CNA-glycine **M15** WAK3583		imidacloprid-5-keto-urea **M33** (DIJ10048)
	imidacloprid-PEDA **M22** DIJ 9646-2		NTN37572
	imidacloprid-AMCP **M16** GSE1478		imidacloprid-formyl-AMCP **M40** (GSE2712)

Table A6.15.3/01-1 Reference substances used for identification of metabolites, continued

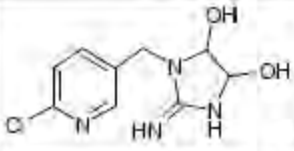
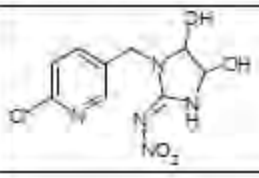
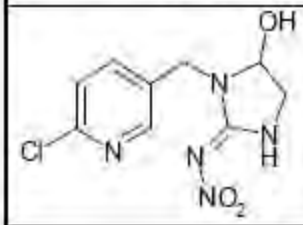
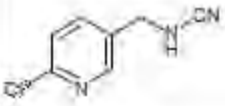
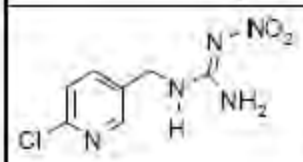
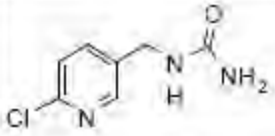
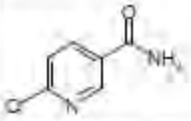
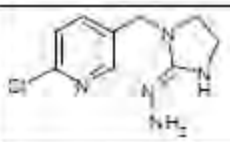
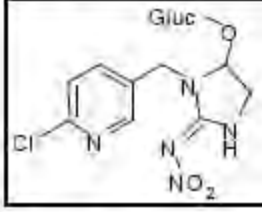
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid-dihydroxyguanidine **M17** WAK 5031		imidacloprid-dihydroxy **M03** WAK 3772
	imidacloprid-5-hydroxy **M04** WAK 4103		imidacloprid-ring-open-guanidine **M24** WAK 4613/3
	imidacloprid-ring-open-nitroguanidine **M11** WAK 4230		imidacloprid-ring-open-urea **M13** DIJ 10739
	6-chloro-nicotinic amide BNF 5518 B		imidacloprid-amino-guanidine **M08** WAK 3877/4
	imidacloprid-5-hydroxy-glucoronide **M04** WAK 4103-Glucuronide		

Table A6.15.3/01-2 Pattern of excretion of a lactating goat after repeated oral administration of 10 mg of imidacloprid per kg b.w. and per day.

Time after dosage [hrs]	Administration No.	Urine [%]	Faeces [%]	Milk [%]	Total [%]
0	1			–	
8		11.84	–	0.078	
24	2	4.64	2.85	0.009	
32		14.74	–	0.079	
48	3	8.50	6.77	0.014	
50	sacrifice	-	–	0.045	
	Subtotal [%]	39.72	9.62	0.225	49.565
	Estimated total residue in edible tissues				5.52
	Total recovery				55.09

Table A6.15.3/01-3 Level of radioactivity in the plasma of a lactating goat

Time after first dosage [hrs]	equivalent concentration [µg/mL]
0.25	1.15
0.5	2.57
1	3.61
2	3.98
3	3.55
4	3.01
6	2.29
8	1.54
24	0.17

Figure A6.15.3/01-1 Level of radioactivity in the plasma of a lactating goat

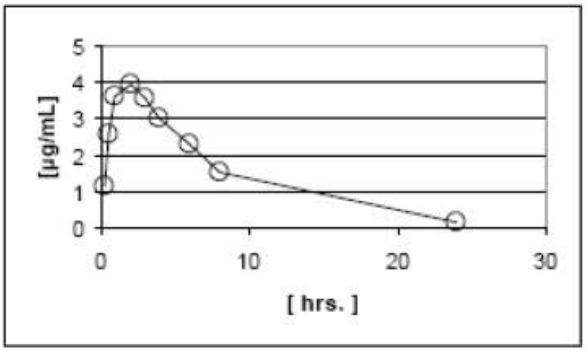


Table A6.15.3/01-4 Radioactivity in milk of a lactating goat after repeated oral administration of 10 mg/kg bw

Time after 1st administration [hrs.]	Administration No.	Milk amount [g]	Equivalent concentration [µg/g]	% of total dose
0	1	--	--	--
8		355	2.09	0.078
24		475	0.17	0.009
24 PA	2	-.	--	--
32		284	2.62	0.079
48		565	0.24	0.014
48 PA	3	--	--	--
50		103	4.10	0.045
Total				0.225

PA = immediately prior to administration

Table A6.15.3/01-5 Residue levels of imidacloprid in the edible tissues and organs of a lactating goat after repeated oral administration (3 x 10 mg/kg bw)

Organ / Tissue	Fresh weight [g]	Equivalent concentration [µg/g]	% of radioactivity, totally administered
Muscle (round)	4.13**	3.96	n.a.
Muscle (flank)	3.69**	3.82	n.a.
Muscle (loin)	3.24**	3.80	n.a.
composite muscle*	8442	3.86***	3.45
Fat (perirenal)	0.51**	1.81	n.a.
Fat (subcutaneous)	0.50**	2.10	n.a.
Fat (omental)	0.61**	2.10	n.a.
Composite Fat*	3377	2.40***	0.73
Liver	725.8	15.92	1.22
Kidney	94.8	11.59	0.12

n.a. = not applicable

* The total weights for muscle and fat in the goat were calculated from the body weight: assuming 30 % for muscle and 12 % for fat for a typical goat. In this case, the animal weight at sacrifice was 28.1 kg.

** mean weight of three samples

*** mean concentration value of the three different types of muscle or fat

Table A6.15.3/01-6 Balance of radioactivity extraction from tissues and organs in % of initial radioactivity

	Kidney	Liver	Muscle			Fat		
			round	flank	loin	perirenal	omental	subcu-taneous
extractable with organic solvents	95.6	94.01	79.7	84.3	89.4	95.3	91.5	96.7
not extractable with water			13.0	8.4	9.0			
solids after extraction with acetonitrile	9.7	6.54	3.2	7.5	2.1	11.2	8.4	8.5
Total	105.3	100.55	95.9	100.2	100.5	106.5	99.9	105.1

Table A6.15.3/01-7 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in milk of the lactating goat after single and repeated oral administration of 10 mg as /kg bw

Dose no.	1				2				3		mean**
Time after 1st administration	8 hrs.		24 hrs. (PA)		32 hrs.		48 hrs. (PA)		50 hrs*		pooled milk
	[µg/g]	%	[µg/g]	%	[µg/g]	%	[µg/g]	%	[µg/g]	%	[µg/g]
Total equivalent concentration	2.09	100	0.17	100	2.62	100	0.24	100	4.10	100	
imidacloprid	0.93	44.6	0.015	8.8	1.08	41.3	0.03	12.5	2.27	55.3	0.50
imidacloprid-5-hydroxy (M01)	0.19	9.0	0.0047	2.7	0.26	9.8	0.010	4.4	0.29	7.0	0.10
imidacloprid-4-hydroxy (M02)	0.24	11.3	0.0056	3.2	0.27	10.2	0.0086	3.6	0.40	9.7	0.12
imidacloprid-olefine (M06)	0.20	9.5	0.0088	5.1	0.23	8.9	0.014	5.8	0.23	5.6	0.097
imidacloprid-nitrosimine (M07)	0.073	3.5	0.005	0.3	0.058	2.2	0.0014	0.6	0.012	0.3	0.025
imidacloprid-6-CNA-glycine (M15)	0.066	3.2	0.011	6.6	0.13	4.8	-	-	0.13	3.1	0.044
Total, identified	1.70	81.1	0.046	26.7	2.03	77.2	0.064	26.9	3.33	81.0	0.89

PA: immediately prior to administration
*: at sacrifice, 2 h after 3rd administration
**: weighted average µg/g
***: parent compound equivalent concentration
%: percent of total recovered radioactivity

Table A6.15.3/01-8 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in kidney and liver of a lactating goat after repeated oral administration of 10 mg as /kg bw

Parent compound/ metabolites	Kidney		Liver	
	%	µg/g	%	µg/g
imidacloprid	5.9	0.68	0.13	0.79
imidacloprid-5-hydroxy (M01)	5.6	0.65		
imidacloprid-4-hydroxy (M02)	2.9	0.34		
imidacloprid-5-hydroxy-glucoronide (M04)	5.7	0.66		
imidacloprid-olefine (M06)	4.3	0.49		
imidacloprid-nitrosimine (M07)	0.1	0.01		
imidacloprid-desnitro/ imidacloprid-ring-open-guanidine (M09/M10)			10.03	1.59
imidacloprid-urea (M12)			0.04	0.01
imidacloprid-6-CNA (M14)			1.53	0.25
imidacloprid-6-CNA-glycine (M15)	13.2	1.53	1.78	0.29
M22			0.21	0.04
Total, identified	37.7	4.36	14.38	2.31

% = percent of total recovered radioactivity (TRR)

Table A6.15.3/01-9 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in organs and tissues of a lactating goat after repeated oral administration of 10 mg as/kg bw

Parent compound/	Muscle						Fat					
metabolites	round		flank		loin		perirenal		omental		subcutaneous	
	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g	%	µg/g
imidacloprid	64.0	2.54	64.5	2.47	68.9	2.65	67.6	1.22	63.4	1.40	73.5	1.54
imidacloprid-5-hydroxy (M01)	3.4	0.13	3.5	0.13	3.2	0.12	3.5	0.07	4.2	0.09	3.1	0.07
imidacloprid-4-hydroxy (M02)	5.7	0.23	5.8	0.22	7.1	0.27	7.0	0.13	8.2	0.18	5.8	0.12
imidacloprid-olefine (M06)	4.9	0.19	5.6	0.21	6.1	0.23	7.6	0.14	10.9	0.22	7.9	0.17
imidacloprid-nitrosimine (M07)	0.25	0.01	0.75	0.03	0.6	0.02	1.0	0.02	-	-	0.6	0.01
Total, identified	78.25	3.10	80.15	3.06	86.85	3.29	86.7	2.58	85.8	1.89	90.9	1.91

%: percent of total recovered radioactivity (TRR)

Section A6.15.3/02

Annex Point IIIA, XI.1.4

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

Metabolism study in the lactating goat

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monographB.7.2.1, II A, 6.2.2.1 /02*
	Authors (year)	[redacted]. (1992)
	Title	[Methylene-^{14}C] Imidacloprid: absorption, distribution, excretion, and metabolism in the liver and kidney of a lactating goat – Amendment to report no. PF3731
	Company, report No.	Bayer CropScience AG, Report-No.: PF3760 BES Ref. : M-024202-01-1
	Date	1992-10-11
	Testing facility	[redacted]
	Dates of work	June 2, 1992 to October 7, 1992
	Test substance(s)	Molecule(s): imidacloprid Purity 99.5% Substance(s): [pyridinyl-^{14}C-methylene] NTN 33893 labelled Specific radioactivity 5.7 MBq/mg, radiochemical purity >99%
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

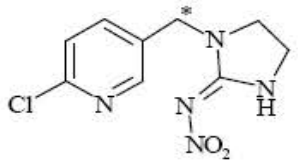
2.1	**Guideline study**	EPA Pesticide Assessment Guidelines Subdivision O, Residue Chemistry, Series 171-4: Nature of the Residue, Livestock (Ruminant) EPA 540/9-82-023, October, 1982
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	[pyridinyl-^{14}C-methylene] NTN 33893 labelled
3.1.1	Lot/Batch number	^{14}C -Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 5.7 MBq/mg, radiochemical purity > 99 %
3.1.2	Specification	

Section A6.15.3/02
Annex Point IIIA, XI.1.4

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

3.1.2.1	Purity	
3.1.2.2	Stability	Stable for the duration of the study
3.1.2.3	Radiolabelling	
3.1.2.4	Reference standards	See Table A6.15.3/02-1
3.2	**Test Animals**	
3.2.1	Species	Bunte deutsche Edelziege, lactating, *(Capra hircus)*
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	about 18 months, weight range 41 kg
3.2.6	Number of animals per group	1
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Concentration of test substance	10 mg/m lin aqueous traganth (0.5%)
3.3.2	Specific activity of test substance	Diluted specificity 0.518 MBq/mg
3.3.3	Volume applied	10 mg/kg bw
3.3.4	Exposure period	3 days
3.3.5	Sampling time	See Table A6.15.3/02-2 for excreta times; tissues and organs at 50h

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

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3.3.7	Metabolite isolation and purification	**Liver:** Liver was suspended in water by means of a tissue homogeniser under ultrasonication. Sediment was separated by centrifugation and re-extracted six times with 0.2 % sodiumchloride solution, followed by extraction with sodium hydroxide–sodium chloride solutions. The extraction residue was lyophilised, weighed and homogenised. Aliquots of this residue were radioassayed by combustion and LSC analysis of combustion gases. The first water extract and the subsequent six 0.2 % sodium chloride solutions were combined and concentrated. After the addition of sodium chloride the sample was taken to complete dryness and the resulting residue suspended in acetonitrile-methanol 2:1 by means of tissue homogeniser. After having separated the supernatant by centrifugation the extraction procedure was repeated four times. During this procedures the recoveries of radioactivity were checked by LSC. The acetonitrile-methanol extracts were combined and concentrated to dryness. The residue was dissolved in water and added with acetonitrile methanol. The fine precipitate was sedimented by centrifugation and the supernatant decanted. The sediment was treated twice in the same way under ultrasonication. The organic phases were combined, concentrated and re-dissolved in water. This solution was subjected to HPLC-analysis. In a second purification step the alkaline sodiumchloride solutions were processed in a similar way as the neutral ones. The resulting aqueous solution was also analysed by HPLC. **Kidney** tissue was suspended in water by means of a tissue homogeniser under ultrasonication. The extract was separated by centrifugation and the sediment was re-extracted five times with 0.2 % sodium chloride solution, followed by extractions with sodium hydroxide–sodium chloride solutions. The balance of radioactivity was checked by LSC. The water extract was combined with the sodium chloride solutions, concentrated to dryness, mixed with acetonitrile – methanol and treated with a tissue homogeniser. The sample was sonicated. The organic solvent was decanted and centrifuged. Remaining sodium chloride was combined with the bulk amount of salt and the extraction procedure was repeated four times. Extracted sodiumchloride and distillates were checked for radioactivity. The acetonitrile-methanol extracts were combined and concentrated. The fine precipitate was sedimented by centrifugation and the supernatant decanted. The sediment was treated twice in the same way under ultrasonication. The organic phases were combined, concentrated and re-dissolved in water. This solution was then three times partitioned with hexane. The volume of the remaining water phase was concentrated and subjected to HPLC-analysis. The hexane phases evaporated to dryness and re-dissolved in water prior to HPLC analysis.

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3.3.8	Metabolite quantitation and identification	**Analysis and identification of metabolites:** RP-HPLC with UV-spectrophotometer and flow-through radioactivity detector. TLC using radioactivity scanning with a linear analyser. Identification of peak components using reference substances with co-chromatography Enzymatic cleavage with ß-glucuronidase/aryl sulfatase **Determination of metabolites with the common moiety 6-chloronicotinic acid:** Small amounts of purified liver and kidney extracts were diluted with water, adjusted to pH 14 and added with an excess amount of potassium permanganate. The oxidation was carried out overnight at 100 °C. After cooling the samples were acidified and extracted with ethyl acetate. The solution was evaporated to dryness and re-constituted in water and subjected to HPLC-analysis. **Measurement of radioactivity:** Measurement of solid samples using LSC: For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in trapped combustion gases was measured by LSC. Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC. Liquid samples were added with scintillation gel and measured by LSC.
		4 RESULTS AND DISCUSSION
4.1	**Absorption and excretion**	The recovery of radioactivity and the excretion pattern after a triple oral administration of 10 mg per day is shown in Table A6.15.3/02-2. The kinetics of total radioactivity was characterised by a fast and predominantly renal elimination of the radioactivity, preceded by an immediate absorption and a rapid distribution process. In total only 0.4 % of the totally administered dose was found in milk. These kinetics also demonstrate that there is no accumulation of radioactivity observable in the milk during the whole test period. Detailed values are given in Table A6.15.3/02-3. The radioactivity levels measured in the samples of tissues and organs as well as the respective weights are given in Table A6.15.3/02-4. The highest equivalent concentration of 17.12 µg/g was determined in the liver, followed by 13.54 µg/g obtained for kidney. This result reflects the significance of these organs for metabolism and excretion of the test compound and its labelled biotransformation products.

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4.2 Metabolism

The successive extraction of liver resulted in more than 99 % of the recovered radioactivity in the extracts. The non-existence of non-extractable radioactivity demonstrated the absence of bound residues in this organ. The total liver radioactive residue was characterised. It contained 68.7 % of the common moiety of 6-chloronicotinic acid.

The overall identification rate in liver was 33.9 % of the organ radioactivity. The two guanidine metabolites imidacloprid-desnitro and imidacloprid-ringopened-guanidine were the main metabolites, accounting for 16.4 % and 7.2 %, respectively.

In kidney the extraction yield was again 99 % of the recovered radioactivity. The extremely low amount of non-extractable material indicated the absence of bound residues also in kidney. Furthermore, 77.9 % of the total radioactive residue was characterised as the common moiety of 6- chloronicotinic acid.

The overall identification rate in kidney was 71.6 % of the organ radioactivity. 5 main metabolites were detected and identified as N33893-olefine (17.7 %), imidacloprid-6-CNA-glycine (M15) (16.8 %), and the glucoronides of the 4- and 5-monohydroxylated metabolites (M04 and M05) (14.06 %). Other metabolites were imidacloprid-desnitro (M09) and imidacloprid-ring-opened guanidine, accounting for 5.9 % and 4.2 %, respectively, as well as imidacloprid (6.2 %). All other metabolites were below the 1 %-level in the kidney. The quantitative distribution of imidacloprid and identified metabolites in kidney and liver and their respective quantities are given in Table A6.15.3/02-5.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

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

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

All samples were radioassayed. Metabolites were extracted from liver and kidney, purified and identified using appropriate analytical techniques.

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5.2 Results and discussion

The excretion amounted to about 58 % of the radioactivity totally administered until sacrifice. The excretion with the urine was the predominant route of elimination, accounting for about 46 % of the dose. The faecal excretion was low with about 12 % of the total dose. A low amount of 0.4 % of the total dose was secreted with the milk. At sacrifice, 2 hours after the final administration, the total residue in the edible organs was estimated to be about 5 %. Based on these values, the total recovery amounted to about 63 %. From the short period after the last dosage it is understandable that at sacrifice the remaining amount of about 40 % was not yet eliminated. Taking into account the low quantities of radioactivity in organs and edible tissues, the recovery indicates that the major portion of the final dose was still present mainly in the digestive tract.

The kinetics of total radioactivity was characterised by a fast and predominantly renal elimination of the radioactivity, preceded by an immediate absorption and a rapid distribution process.

Comparable equivalent concentrations were measured in milk eight hours after the first and the second (32 hrs.) administration with values of 3.2 µg/g and 2.8 µg/g. At 24 hrs. after the 2nd administration the concentration in the milk was reduced by factors of 14 to a value of 0.2 µg/mL. The highest equivalent concentration of 3.6 µg/g was determined 2 hours after the third application. In total only 0.4 % of the totally administered dose was found in milk. These kinetics also demonstrate that there is no accumulation of radioactivity observable in the milk during the whole test period. In terms of amounts, only 0.4 % of the dose administered was found in the milk until the end of the test.

.The total compound related residues in the body musculature amounted to 3.65 % of the total administered radioactivity, assuming that the musculature accounted for 30 % of body weight. Even lower quantities were determined in the different fat tissues. The mean value was 1.07 µg/g which corresponds to 0.73 % of the totally administered radioactivity. (It was assumed that the fat tissues accounted for 12 % of the body weight.) The highest equivalent concentration of 17.12 µg/g was determined in the liver, followed by 13.54 µg/g obtained for kidney. This result reflects the significance of these organs for metabolism and excretion of the test compound and its labelled biotransformation products.

The successive extraction of liver resulted in more than 99 % of the recovered radioactivity in the extracts. The non-existence of non-extractable radioactivity demonstrated the absence of bound residues in this organ. The total liver radioactive residue was characterised. It contained 68.7 % of the common moiety of 6-chloronicotinic acid.

The overall identification rate in liver was 33.9 % of the organ radioactivity. The two guanidine metabolites imidacloprid-desnitro and imidacloprid-ringopen-guanidine were the main metabolites, accounting for 16.4 % and 7.2 %, respectively.

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5.2 continued

In kidney the extraction yield was again 99 % of the recovered radioactivity. The extremely low amount of non-extractable material indicated the absence of bound residues also in kidney. Furthermore, 77.9 % of the total radioactive residue was characterised as the common moiety of 6- chloronicotinic acid.

The overall identification rate in kidney was 71.6 % of the organ radioactivity. 5 main metabolites were detected and identified as N33893-olefine (17.7 %), imidacloprid-6-CNA-glycine (M15) (16.8 %), and the glucoronides of the 4- and 5-monohydroxylated metabolites (M04 and M05) (14.06 %). Other metabolites were imidacloprid-desnitro (M09) and imidacloprid-ring- openguanidine, accounting for 5.9 % and 4.2 %, respectively, as well as imidacloprid (6.2 %). All other metabolites were below the 1 %-level in the kidney.

5.3 Conclusion for both A6.15.3/01 and 02

The radioactivity in the plasma of a lactating goat reached the highest concentration at 2 hours after the first oral administration of [pyridinyl-14C-methylene]-imidacloprid with an equivalent concentration of 3.98 µg/mL, corresponding to about 40 % of the equidistribution concentration in the body. The radioactivity was eliminated from the plasma with a half-life of about 4.8 hours for the time period from 2 to 24 hours after the first administration. Thus, the absorption, distribution and elimination of imidacloprid was a rather fast process. Within 50 hours after the first administration the excretion amounted to about 54 % of the radioactivity totally administered until sacrifice. The excretion with the urine was the predominant route of elimination, accounting for about 43 % of the dose. The faecal excretion was low with about 11 % of the total dose. A negligible amount of 0.3 % of the total dose was secreted with the milk. At sacrifice, 2 hours after the final administration, the total residue in the edible organs was estimated to be about 5 %. Based on these mean values from both studies, the total recovery amounted to about 65 %.

The metabolisation rate of imidacloprid in kidney and liver was very high but in muscle and fat tissues about 65 % were identified as imidacloprid. Three main routes of the degradation of imidacloprid in goats were identified:
- Hydroxylation of the imidazolidine ring of imidacloprid produced 4-hydroxy and 5-hydroxy imidacloprid (M01, M02) plus the glucoronide conjugate of the monohydroxy metabolites (M04, M05), and the dihydroxy imidacloprid (M03). Dehydration of 4-hydroxy and 5-hydroxy imidacloprid (M01, M02) leads to imidacloprid-olefine (M06).
- Reduction and loss of the nitro group gives rise to imidacloprid-amino-guanidine (M08) and imidacloprid-desnitro (M09) and finally by hydrolysis imidacloprid-urea (M12).
- Opening of the imidazolidine ring by removal of the ethylene bridge and subsequent oxidation leads to imidacloprid-ring-open nitro-guanidine (M11) and imidacloprid-ring open guanidine (M10) which can also be formed from both imidacloprid-desnitro (M09) and imidaclopriddihydroxyguanidine (M17). imidacloprid-ring-open-guanidine (M10) can form imidaclopridring- open-urea (M13) and imidacloprid-AMCP (M16). Further degradation leads to 6 chloro-nicotinic acid (M14), which conjugates with glycine (M15).

Looking across both studies, it can be concluded that the metabolisation

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		of imidacloprid in lactating goat is very high. Only approximately 0.3% of the total dose was excreted in milk. Approximately 50% of the radioactive residue in milk was identified as imidacloprid 8 hours after administration but only 10% as parent 24 hours after an administration. At sacrifice 2 hours after final administration the total residue in edible organs was estimated to be about 5%. Parent compound, imidacloprid olefin and imidacloprid 5-hydroxy formed most of the residue detected. The proposed pathway of imidacloprid in the lactating goat is depicted in Figure A6.15.3/02-1.
5.3.1	Reliability	1
5.3.2	Deficiencies	No when combined with A6.15.3/01

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/04/05
Materials and Methods	Acceptable
Results and discussion	The applicant's version is acceptable.
Conclusion	The presented metabolism study in lactating goats has been previously described in the imidacloprid DAR for Annex I inclusion according to Directive 91/414/EEC. Crop use related MRLs for imidacloprid in EU Member States have been defined and the proposed residue definition for risk assessment is "imidacloprid including the degradation and reaction products, determined as 6-chloronicotinic acid; calculated as imidacloprid", the residue definition for monitoring is "sum of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, expressed as imidacloprid". Measurable residues in food or feed from the use of imidacloprid in PT18 biocidal products are not expected. Therefore, an additional exposure to humans through diet arising from the use of imidacloprid as a biocide can be excluded. No MRLs specific to biocidal product uses are necessary.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.15.3/02-1 Reference substances used for identification of metabolites

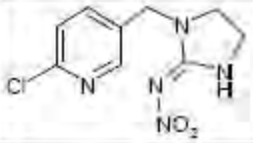
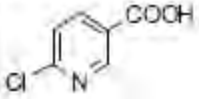
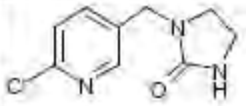
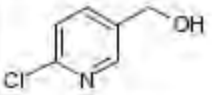
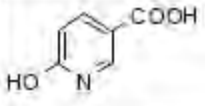
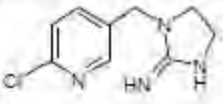
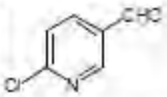
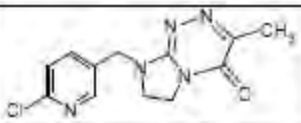
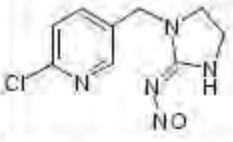
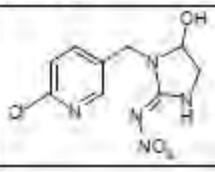
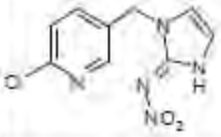
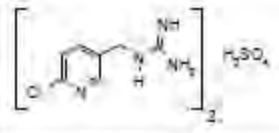
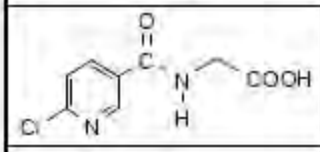
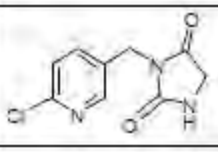
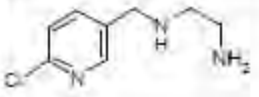
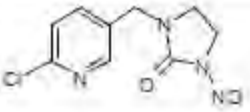
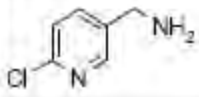
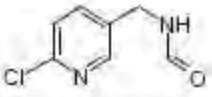
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid as		imidacloprid-6-CNA **M14**
	imidacloprid-urea **M12** (NTN33519)		imidacloprid-CHMP **M28** (DIJ9805)
	6-hydroxy-nicotinic acid (**M18**) (GBH4315)		imidacloprid-desnitro NTN38014 **M09**
	MAT 10249-0		imidacloprid-triazinone **M25**
	imidacloprid-nitrosimine **M07** (WAK3839)		imidacloprid-5-hydroxy **M01** (WAK4103)
	imidacloprid-olefine **M06** (NTN 35884)		imidacloprid-ring-open-guanidine **M10** (WAK4126)
	imidacloprid-6-CNA-glycine **M15** WAK3583		imidacloprid-5-keto-urea **M33** (DIJ10048)
	imidacloprid-PEDA **M22** DIJ 9646-2		NTN37572
	imidacloprid-AMCP **M16** GSE1478		imidacloprid-formyl-AMCP **M40** (GSE2712)

Table A6.15.3/02-1 Reference substances used for identification of metabolites, continued

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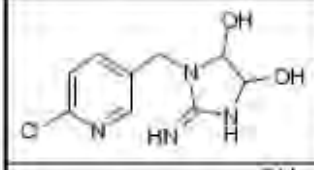
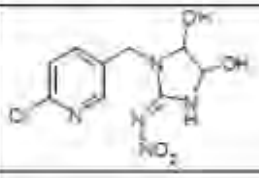
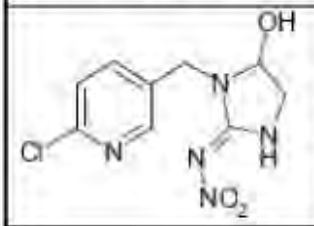
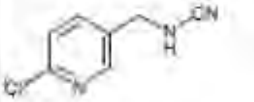
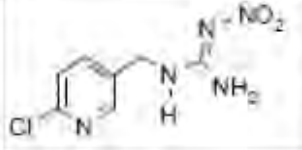
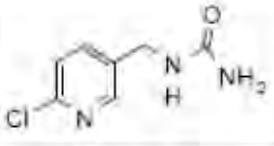
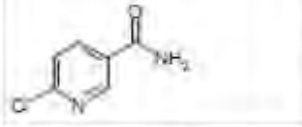
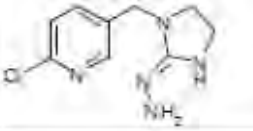
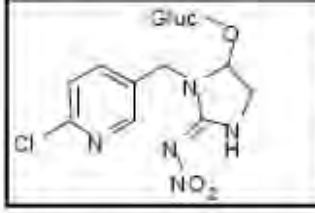
	imidacloprid-dihydroxyguanidine **M17** WAK 5031		imidacloprid-dihydroxy **M03** WAK 3772
	imidacloprid-5-hydroxy **M04** WAK 4103		imidacloprid-ring-open-guanidine **M24** WAK 4613/3
	imidacloprid-ring-open-nitroguanidine **M11** WAK 4230		imidacloprid-ring-open-urea **M13** DIT 10739
	6-chloro-nicotinic amide BNF 5518 B		imidacloprid-amino-guanidine **M08** WAK 3877/4
	imidacloprid-5-hydroxy-glucoronide **M04** WAK 4103-Glucuronide		

Table A6.15.3/02-2 Pattern of excretion of a lactating goat after repeated oral administration of 10 mg of imidacloprid per kg b.w. and per day.

Time after dosage [hrs]	Administration No.	Urine [%]	Faeces [%]	Milk [%]	Total [%]
0	1			--	
8			--	0.131	
24	2	19.76	2.39	0.022	
32			--	0.187	
48	3	21.19	6.65	0.026	
50	sacrifice	5.08	2.53	0.046	
	Subtotal [%]	46.03	11.57	0.41	58.01
	Estimated total residue in edible tissues				5.27
	Total recovery				63.28

Table A6.15.3/02-3 Radioactivity in milk of a lactating goat after repeated oral administration of 10 mg as/kg bw

Time after 1st administration (h)	Administration no.	Milk amount (g)	Equivalent concentration (µg/g)	% of total dose
0	1	-	-	-
8		512	3.16	0.131
24 (PA)		1458	0.19	0.022
24	2	-	-	-
32		834	2.77	0.187
48 (PA)		1627	0.20	0.026
48	3		-	-
50	(sacrifice)	156	3.65	0.046
Total				0.413

PA = immediately prior to administration

Table A6.15.3/02-4 Residue levels of imidacloprid in the edible tissues and organs of a lactating goat after repeated oral administration (3 x 10 mg/kg bw)

Organ / Tissue	Fresh weight (g)	Equivalent concentration (µg/g)	% of radioactivity, totally administered
Liver	936.05	17.12	1.30
Kidney	120.25	13.54	0.13
Muscle (round)	2555.42	3.33	-
Muscle (flank)	681.97	3.62	-
Muscle (loin)	184.00	3.68	-
Total body musculature*	11580.00	3.65**	3.44
Fat (perirenal)	224.98	0.92	-
Fat (subcutaneous)	220.33	1.19	-
Fat (omental)	629.32	0.94	-
Total body fat*	4632.00	1.07**	0.40

-: Not applicable

*: The total weights for muscle and fat in the goat were calculated from the body weight: assuming 30 % for muscle and 12 % for fat for a typical goat. In this case, the animal weight at sacrifice was 38.6 kg.

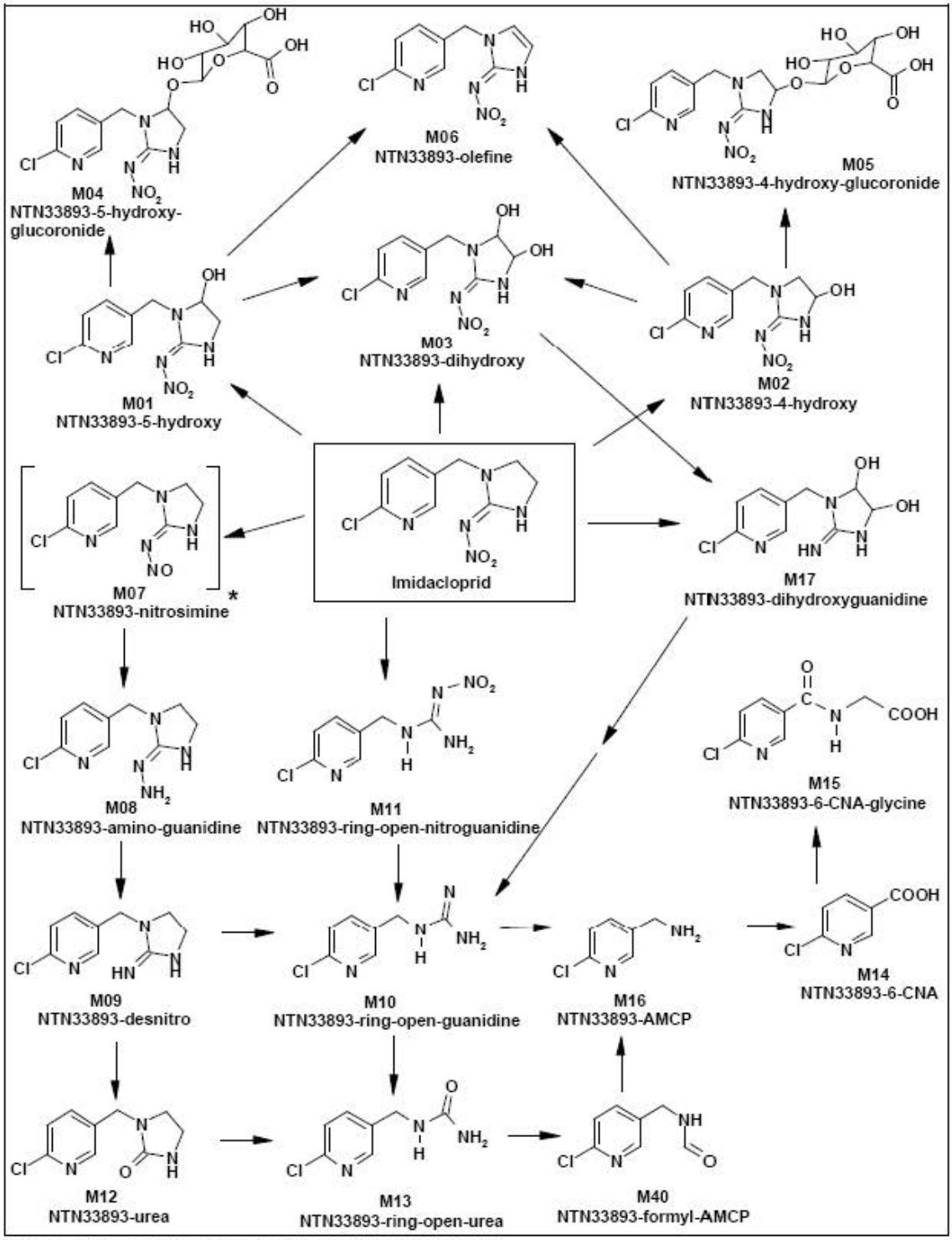
**: Mean concentration value of the three different types of muscle or fat

Table A6.15.3/02-5 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in kidney and liver of a lactating goat after repeated oral administration of 10 mg as /kg bw

Parent compound/ metabolites		Kidney		Liver	
		%	[µg/g]	%	[µg/g]
Total recovered radioactivity			13.53		17.11
imidacloprid		6.19	0.838		
imidacloprid-5-hydroxy (M01)	WAK 4103	1.96	0.265		
imidacloprid-4-hydroxy (M02)	WAK 5839				
imidacloprid-dihydroxy (M03)	WAK 3772				
M04/	imidacloprid-5-hydroxy-glucoronide	14.06	1.904		
M05	imidacloprid-4-hydroxy-glucoronide				
imidacloprid-olefine (M06)	NTN 35884	17.71	2.398	3.17	0.543
imidacloprid-amino-guanidine (M08)	WAK 3877/4			1.52	0.260
imidacloprid-desnitro (M09)	NTN 38014	5.86	0.793	16.39	2.806
imidacloprid-ring-open-guanidine (M10)	WAK 4126	4.19	0.567	7.23	1.238
imidacloprid-ring-open-nitroguanidine (M11)	WAK 4230	0.81	0.110	0.35	0.060
imidacloprid-urea (M12)	NTN 33519	0.73	0.099	1.96	0.336
imidacloprid-ring-open-urea (M13)	DIJ 10739	0.19	0.026	1.26	0.216
imidacloprid-5-CNA (M14)	6-CNA	0.32	0.043		
imidacloprid-6-CNA-glycine (M15)	WAK 3583	16.78	2.272	0.96	0.164
imidacloprid-AMCP (M16)	GSE 1478	1.84	0.249	0.43	0.074
imidacloprid-dihydroxy-guanidine (M17)	WAK 5031	0.61	0.083	0.60	0.103
M40	GSE 2712	0.37	0.050		
Sum identified		71.62	9.697	33.87	5.800
6-CNA containing residues (GC)		77.92	10.550	68.67	11.756

% = percent of total recovered radioactivity (TRR)

Figure A6.15.3/02-1: Proposed pathway of imidacloprid in lactating goat



Notes: For codes and names see also the list of metabolites.

*) imidacloprid-nitrosimine was only found in in the first lactating goat study, (Karl, Klein, Weber, 1991) in traces. In the second lactating goat study (Klein, 1992) it was not detected.

Section A6.15.3/03

Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

Official use only

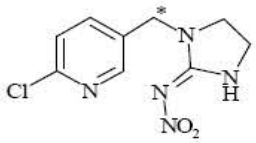
1 REFERENCE

1.1	Reference	*PPP monographB.7.2.2, II A, 6.2.2.2 /01*
	Authors (year)	[redacted] (1990)
	Title	(Methylene-^{14}C) imidacloprid - Absorption, distribution, excretion and metabolism in laying hens
	Company, report No.	Bayer CropScience AG, Report-No.: PF3558 BES Ref. : M-024187-01-1
	Date	1990-09-17
	Testing facility	[redacted]
	Dates of work	May 24, 1988 to September 17, 1990
	Test substance(s)	Molecule(s): imidacloprid Purity 99.5% Substance(s): [pyridinyl-^{14}C-methylene] NTN 33893 labelled Specific radioactivity 5.7 MBq/mg, radiochemical purity >99%
1.2	Data protection	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

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

3 MATERIALS AND METHODS

3.1	Test material	[pyridinyl-^{14}C-methylene] NTN 33893 labelled
3.1.1	Lot/Batch number	^{14}C -Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 5.7 MBq/mg, radiochemical purity > 99 %
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	Stable in the administraton suspension for at least 4 hours
3.1.2.3	Radiolabelling	
3.1.2.4	Reference standards	See Table A6.15.3/03-1

Section A6.15.3/03

Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

3.2	**Test Animals**	
3.2.1	Species	Species / strain: White Leghorn, laying hens (*Gallus domesticus*)
3.2.2	Strain	Age about 6 to 8 months, Body weight: 1.35 – 1.68 kg (test start), 1.11 – 1.65 kg (sacrifice)
3.2.3	Source	Number of animals: 18 plus 9 reserve animals, 3 groups with five hens plus 3 reserve in each.
3.2.4	Sex	First group: untreated control group, used as blank samples.
3.2.5	Age/weight at study initiation	Second group: Radioactively dosed hens, to be used for determining the maximum plasma level and later substituted by reserve group.
3.2.6	Number of animals	Third group: Radioactively dosed hens, used for the metabolism investigation. A fourth group had to be included in the study since unexpected toxic symptoms occurred in the second group. The dose was reduced from the target dose of 50 mg/kg to 10 mg/kg in the third group and in this dose group which was used to determine the maximum plasma level.
3.3	**Administration/ Exposure**	Oral
3.3.1	Concentration of test substance	Compound was suspended in 0.5 % tragacanth suspension. The calibration of the administration suspension resulted in an average value of 252.1 µCi (= 9.33 MBq) per animal and dosage in test No. 2 and 261.59 µCi (= 9.68 MBq) in test No.3 and 4.
3.3.2	Specific activity of test substance	
3.3.3	Volume applied	The labelled material was orally administered to animal group No. 2 as a 0.5 % suspension in tragacanth once daily at a target dose level of 50 mg per kg of bw. Since toxic symptoms became obvious during the test, the target dose was reduced to a target dose of 10 mg per kg of bw for the dose groups No. 3 and No. 4 as shown in Table A6.15.3/03-2.
3.3.4	Exposure period	3 days
3.3.5	Sampling time	See Table A6.15.3/01-3 for excreta times; tissues and organs at 50h

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

Metabolism study in the laying hen

3.3.6 Samples

To determine the time course of the plasma concentration, microsamples of blood (about 60 µL) were taken from the wing veins of the hens at 0.25, 0.5, 1, 2, 3, 4, 6 and 24 hours after the third administration from the animal group no. 2.

Eggs of test no. 2 and 3 were collected twice daily, in the morning before the daily administration and 8 hours after administration. Furthermore the eggs were dissected from the oviduct at sacrifice, 2 hours after the last administration. In test no. 4 eggs were only collected during the test phase. Number and weight of eggs were recorded for all hens. For sampling the eggshells were discarded, white and yolk were thoroughly mixed, separately for the eggs collected per day and hen. Subsamples from each egg-mix were radioassayed.

The excreta were individually collected in intervals of 24 hours, immediately prior to each administration.

From animal group 1 and 3 the following tissues and organs were dissected and radioassayed: kidney, liver, heart, gizzard, skin without subcutaneous fat, breast muscle and thigh muscle and subcutaneous fat. The tissues and organs were thoroughly homogenized using a meat mincer after adding some pieces of dry ice. The subsamples of the resulting tissue pulp were weighed, freeze-dried and weighed again. Subsamples were subjected to combustion followed by LSC analysis of combustion gases in the freeze-dried state because the existence of volatile components could be ruled out. In parallel subsamples were radioassayed in the wet state using the BTS-tissue solubiliser.

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

Metabolism study in the laying hen

3.3.7	Metabolite isolation and purification, quantitation and identification	**Excreta:** 40 % of the collected excreta were extracted with water and dichloromethane. Combined and concentrated extracts were separated into several fraction by prep. scale HPLC. Fractions were rechromatographed and finally split into 9 fractions. The main fraction was further separated by analytical scale HPLC for further spectroscopic investigations. **Eggs:** Pooled egg-mix was extracted with acetonitrile-methanol. Extracts were combined, lyophilised and concentrated. These lyophilised samples were re-dissolved in methanol and mixed with RP18- modified silica gel in the batch mode. After ultrafiltration under pressure the sample was extracted with water and water-acetonitrile with increased amounts of acetonitrile. Finally it was extracted with water (buffer pH 2)-acetonitrile. The first extracts were combined and subjected to further purification by HPLC. The acidic acetonitrile extracts were also subjected to micro-prep. HPLC. **Liver:** Liver samples were extracted with water. The extracts were lyophilised and re-extracted with acetonitrile – methanol. The extracts were concentrated and subjected to prep. HPLC. Fractions were re-chromatographed and analysed by H-NMR and mass spectroscopy. **Kidney:** Kidney samples were extracted with water. The extracts were lyophilised and re-extracted with acetonitrile-methanol. The extract was concentrated and subjected to prep. HPLC. This procedure yielded several fractions, which were further separated by micro-prep. HPLC on analytical HPLC columns. **Heart, muscle, gizzard, skin:** Tissue homogenate was extracted with water. The extracts were lyophilised and re-extracted using acetonitrile-methanol. These phases were then analysed by HPLC. **Subcutaneous fat:** Fat was dissolved in acetonitrile – water. After centrifugation the upper organic phase was concentrated and subjected to HPLC. **Analysis and identification of metabolites:** RP-HPLC with UV-spectrophotometer and flow-through radioactivity detector. TLC using radioactivity scanning with an linear analyser. Identification of peak components using reference substances with co-chromatography. H-NMR–spectra were recorded on a 300 MHz spectrometer. Mass spectra were recorded using EI-and CI-ionisation techniques. **Measurement of radioactivity:** Measurement of solid samples using LSC: For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in trapped combustion gases was measured by LSC. Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC. Liquid samples were added with scintillation gel and measured by LSC.

4 RESULTS AND DISCUSSION

4.1 Absorption and excretion

The recovery of radioactivity and the excretion pattern after a triple oral administration of 10 mg per day is shown in Table A6.15.3/03-3. Until sacrifice (50 hrs after the first administration) the excretion amounted to 32.9 % of the radioactivity totally administered. The excretion rate was moderate. About one half of the radioactivity administered with the first dose was eliminated from the body during the first 24 hours. This value is significantly lower than in rats.

Although the excreta of birds represent a mixture of faeces and urine, it can be concluded from the high concentration in the kidneys that the bulk of the radioactivity was excreted renally. This indicates that the quantity absorbed from the gastrointestinal tract was very high and that the biggest part of the last dose was still present in it. A low amount of radioactivity was found in the eggs. At sacrifice, 2 hours after the last administration, the residues in the tissues and organs dissected from the body was calculated and estimated to be 3.4 % of the total dose.

The radioactivity level in the plasma reached the maximum concentration at 2 hours after the third oral administration with an equivalent concentration of 4.90 µg/mL, corresponding to about one half of the equidistribution concentration in the body. This result is a hint on a rapid distribution of the administered radioactivity from the plasma into the peripheral organs and tissues. The radioactivity was eliminated from the plasma with a half-life of about 14 hours for a time period from 6 to 24 hours after the last administration. Based on this short half-life and on the relatively low plasma concentration, for the main residence time (MRT) the value of 21.5 hours were obtained. At the end of this test, 24 hrs. after the third administration, the plasma concentration had declined to a value of 2.09 µg/mL. The plasma concentrations measured in the study are presented in Table A6.15.3/03-4 and depicted in Figure A6.15.3/03-1.

The equivalent concentrations in the eggs of both dosed animal groups obtained during the whole test period were extremely low. There was only a slight increase in concentration with increasing time after the onset of the tests. The concentrations ranged from 0.358 µg/g (lowest value) to 24 hrs. to 0.789 µg/g at the maximum, 48 hrs. after the first dose.

The resulting residue levels of the total radioactivity in the edible tissues and organs at the time of the maximum plasma concentration are given in A6.15.3/03-5

4.2 Metabolism

Identification of radioactive metabolites by means of 1H-NMR- and mass spectroscopy was not possible in all cases due to extremely low concentrations and small amounts of radioactive residues in organs, tissues and eggs. The overall identification or characterisation rate of the residues reached on average of 37.1 %. The biotransformation products detected in organs, tissues and eggs with their respective quantities are listed in Table A6.15.3/03-6

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Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a study conducted according to EPA guideline 171-4, the absorption, distribution, excretion and metabolism of imidacloprid in laying was elucidated after three consecutive daily administrations of radiolabelled test substance administered by intubation of an aqueous solution once daily at a target dose level of first 50 and finally 10 mg/kg bw. The treated animals were sacrificed at plasma peak level as determined after the first administration.

Samples of plasma, eggs and excreta were taken at pre-determined times; organs and tissues (liver, kidney, heart, muscle, gizzard, skin and subcutaneous fat) were taken after sacrifice on day 3.

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

5.2 Results and discussion

Until sacrifice (50 hrs after the first administration) the excretion amounted to 32.9 % of the radioactivity totally administered. About one half of the radioactivity administered with the first dose was eliminated from the body during the first 24 hours, significantly lower than in rats.

The radioactivity level in the plasma reached the maximum concentration at 2 hours after the third oral administration with an equivalent concentration of 4.90 µg/mL, corresponding to about one half of the equidistribution concentration in the body. The radioactivity was eliminated from the plasma with a half-life of about 14 hours for a time period from 6 to 24 hours after the last administration. Based on this short half-life and on the relatively low plasma concentration, for the main residence time (MRT) the value of 21.5 hours were obtained. At the end of this test, 24 hrs. after the third administration, the plasma concentration had declined to a value of 2.09 µg/mL.

It can be concluded from the high concentration in the kidneys that the bulk of the radioactivity was excreted renally. This indicates that the quantity absorbed from the gastrointestinal tract was very high and that the biggest part of the last dose was still present in it. At sacrifice , the residues in the tissues and organs dissected from the body was calculated and estimated to be 3.4 % of the total dose.

The equivalent concentrations in the eggs of both dosed animal groups obtained during the test period were extremely low. There was only a slight increase in concentration with increasing time after the onset of the tests. The concentrations ranged from 0.358 µg/g (lowest value) to 24 hrs. to 0.789 µg/g at the maximum, 48 hrs. after the first dose.

The resulting residue levels of the total radioactivity in the edible tissues and organs were very low. Therefore, identification of metabolites was not possible in all cases. Parent compound and metabolite M(06), imidacloprid olefin, were identified in several samples. Desnitro-imidacloprid, M(09) , was observed in kidney.

5.3 Conclusion

The overall conclusion of poultry metabolism can be found in the conclusion section of A6.15.3/04.

Section A6.15.3/03 Annex Point IIIA, XI.1.4	Estimation of exposure to humans or animals through food and feeding stuffs and other means *Metabolism study in the laying hen*
5.3.1 Reliability	1
5.3.2 Deficiencies	No, with the understanding further efforts on elucidating metabolism are reported in study summary A6.15.3/04

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/04/05
Materials and Methods	Acceptable
Results and discussion	The applicant's version is acceptable.
Conclusion	See also point A6.15.3/04. The presented metabolism study in laying hen has been previously described in the imidacloprid DAR for Annex I inclusion according to Directive 91/414/EEC. Crop use related MRLs for imidacloprid in EU Member States have been defined and the proposed residue definition for risk assessment is "imidacloprid including the degradation and reaction products, determined as 6-chloronicotinic acid; calculated as imidacloprid", the residue definition for monitoring is "sum of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, expressed as imidacloprid". Measurable residues in food or feed from the use of imidacloprid in PT18 biocidal products are not expected. Therefore, an additional exposure to humans through diet arising from the use of imidacloprid as a biocide can be excluded. No MRLs specific to biocidal product uses are necessary.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.15.3/03-1 Reference substances used for identification of metabolites

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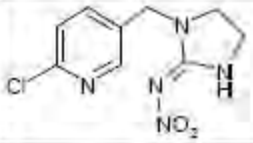
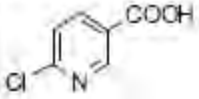
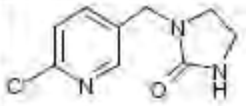
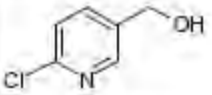
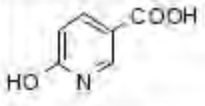
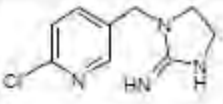
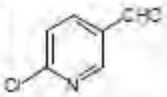
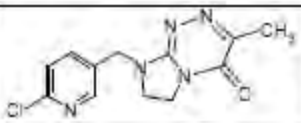
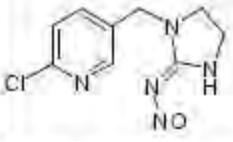
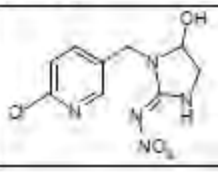
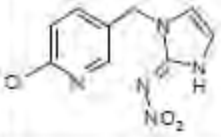
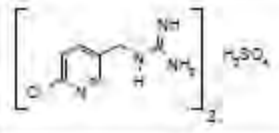
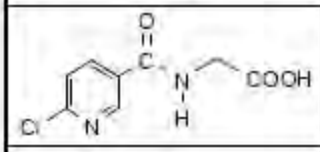
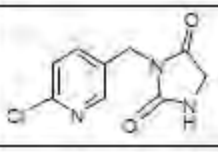
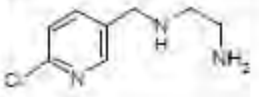
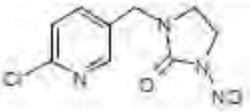
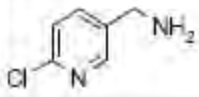
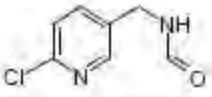
	imidacloprid as		imidacloprid-6-CNA **M14**
	imidacloprid-urea **M12** (NTN33519)		imidacloprid-CHMP **M28** (DIJ9805)
	6-hydroxy-nicotinic acid (**M18**) (GBH4315)		imidacloprid-desnitro NTN38014 **M09**
	MAT 10249-0		imidacloprid-triazinone **M25**
	imidacloprid-nitrosimine **M07** (WAK3839)		imidacloprid-5-hydroxy **M01** (WAK4103)
	imidacloprid-olefine **M06** (NTN 35884)		imidacloprid-ring-open-guanidine **M10** (WAK4126)
	imidacloprid-6-CNA-glycine **M15** WAK3583		imidacloprid-5-keto-urea **M33** (DIJ10048)
	imidacloprid-PEDA **M22** DIJ 9646-2		NTN37572
	imidacloprid-AMCP **M16** GSE1478		imidacloprid-formyl-AMCP **M40** (GSE2712)

Table A6.15.3/03-1 Reference substances used for identification of metabolites, continued

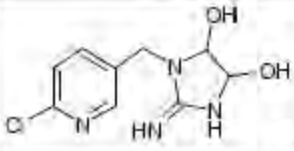
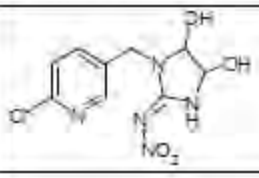
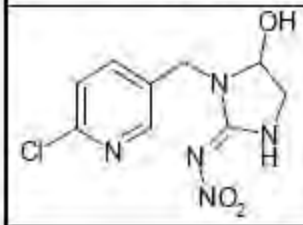
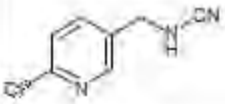
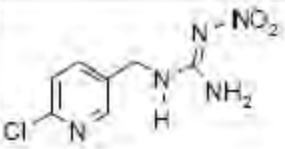
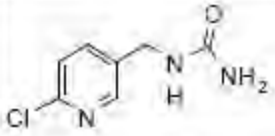
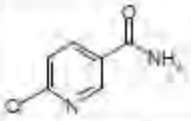
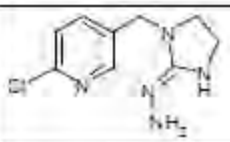
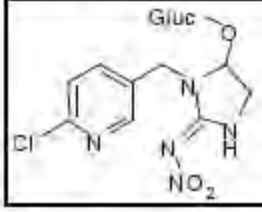
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid-dihydroxyguanidine **M17** WAK 5031		imidacloprid-dihydroxy **M03** WAK 3772
	imidacloprid-5-hydroxy **M04** WAK 4103		imidacloprid-ring-open-guanidine **M24** WAK 4613/3
	imidacloprid-ring-open-nitroguanidine **M11** WAK 4230		imidacloprid-ring-open-urea **M13** DIJ 10739
	6-chloro-nicotinic amide BNF 5518 B		imidacloprid-amino-guanidine **M08** WAK 3877/4
	imidacloprid-5-hydroxy-glucoronide **M04** WAK 4103-Glucuronide		

Table A6.15.3/03-2 Administration of [pyridinyl-^{14}C methylene] imidacloprid to laying hens

Test group No.	Number of animals	Dilution factor with unlabelled compound	Mean dose per animal [µCi]	Mean Dose [mg/kg bw]	Remarks
1	5	-	-	-	untreated control
2	5	1:50	252.10	52.9	determination of plasma peak level *)
3	5	1:10	261.59	10.6	metabolism investigation
4	3	1:10	261.59	10.6	determination of plasma peak level *)

Note: *) Since the dose level was not tolerated without toxic symptoms, test No. 4 was included in the study

Table A6.15.3/03-3 Percentages of the total radioactivity excreted/secreted with excreta and egg

Sample	Time after 1st application [hrs]	[%]
Excreta	24	47.3 ± 2.3
	50	21.3 ± 0.8
Subtotal		32,9 ± 0.08
Eggs*)	50	0.062 ± 0.008
Calculated residues in the tissues	50	3.39 ± 1.10

Note: *) incl. eggs dissected from the oviduct at sacrifice

Table A6.15.3/03-4 Level of radioactivity in the plasma (mean values) as a function of time

Time after 3rd dosage [hours]	[µg/mL]	% CV
1	4.31	16
2	4.90	4
4	4.77	17
6	5.00	17
8	4.75	18
24	2.09	18

Table A6.15.3/03-5 Equivalent concentration in the dissected tissues and organs of laying hens at the time of sacrifice, 50 hours after the first administration

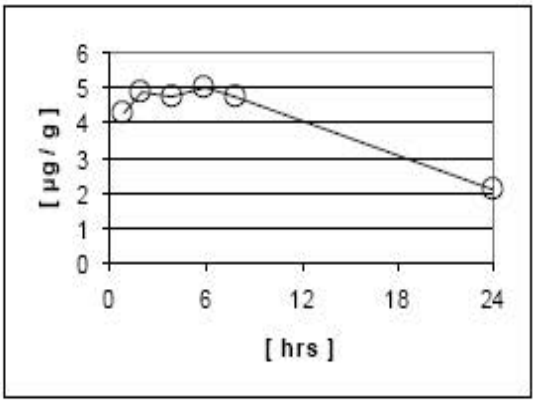
Tissue/organ	Equivalent concentration Mean ± SDVE* µg/g
Liver	8.158 ± 1.17
Kidney	11.522 ± 2.699
Heart	3.182 ± 1.583
Gizzard without lining and contents	6.486 ± 3.632
Muscle, thigh	1.484 ± 0.297
Muscle, breast	2.353 ± 1.713
Fat, subcutaneous	0.455 ± 0.153
Skin without fat	1.250 ± 0.310
Eggs (after 24 hrs)	0.358
Eggs (after 48 hrs.)	0.789

*Arithmetic mean ± standard deviation

Table A6.15.3/03-6 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in organs, tissues and eggs of a laying hens 50 hours after repeated oral administration of 3 x 10 mg as/kg bw

Parent compound/ metabolite	Eggs µg/g	Kidney µg/g	Breast µg/g	Heart µg/g	Gizzard µg/g	Skin µg/g	Thigh muscle µg/g	Fat µg/g
Imidacloprid	-	-	1.07	0.88	3.43	0.09	0.08	0.49
imidacloprid-olefine (M06)	0.22	0.69	-	0.64	-	0.35	0.43	-
imidacloprid-desnitro (M09)	-	0.41	-	-	-	-	-	-

Figure A6.15.3/03-1 Level of radioactivity in the plasma (mean values) as a function of time



Section A6.15.3/04

Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

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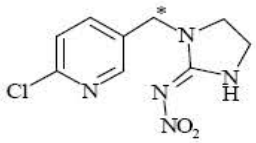
1 REFERENCE

1.1	Reference	*PPP monographB.7.2.2, II A, 6.2.2.2 /02*
3.	Authors (year)	[redacted] (1992)
	Title	[Methylene-^{14}C] Imidacloprid: Absorption, distribution, excretion, and metabolism in laying hens – Amendment to report no. PF3558
	Company, report No.	Bayer CropScience AG, Report-No.: PF3759 BES Ref. : M-024216-01-1
	Date	1992-09-16
4.	Testing facility	[redacted]
5.	Dates of work	March 10, to September 16, 1992
6.	Test substance(s)	Molecule(s): imidacloprid Purity 99.8% Substance(s): [pyridinyl-^{14}C-methylene] NTN 33893 labelled Specific radioactivity 4.11 MBq/mg, radiochemical purity >99%
1.2	Data protection	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

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

3 MATERIALS AND METHODS

3.1	Test material	[pyridinyl-^{14}C-methylene] NTN 33893 labelled
3.1.1	Lot/Batch number	^{14}C-Labelled: [methylene-^{14}C]-imidacloprid, specific radioactivity 4.11 MBq/mg, radiochemical purity > 99 %
3.1.2	Specification	
3.1.2.1	Purity	
3.1.2.2	Stability	Stable in the administration suspension for at least 4 hours
3.1.2.3	Radiolabelling	
3.1.2.4	Reference standards	See Table A6.15.3/04-1

Section A6.15.3/04

Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

3.2	**Test Animals**	
3.2.1	Species	Species / strain: White Leghorn, laying hens (*Gallus domesticus*) Age about 6 to 8 months, Body weight: 1.34 – 1.61 kg (test start) Number of animals: five hens
3.2.2	Strain	
3.2.3	Source	
3.2.4	Sex	
3.2.5	Age/weight at study initiation	
3.2.6	Number of animals	
3.3	**Administration/ Exposure**	Oral
3.3.1	Concentration of test substance	In order to prepare the administration solution, the compound was suspended in 0.5 % tragacanth suspension. The radioactive test compound was diluted with non-labelled compound by a ratio of 1:10. The calibration of the administration suspension resulted in an average value of 181.989 µCi (=6.73 MBq) per animal and dosage. The labelled material was orally administered as a 0.5 % suspension in tragacanth once daily at a target dose level of 10 mg per kg of bw.
3.3.2	Specific activity of test substance	
3.3.3	Volume applied	
3.3.4	Exposure period	3 days
3.3.5	Sampling time	See Table A6.15.3/04-2 for excreta times; tissues and organs at 50h
3.3.6	Samples	Eggs were collected during the accommodation and test period twice daily. Assuming a laying rate of 290 eggs per hen and year at the maximum, the overall egg production observed with three animals during both periods was ca. 76 %. Reasons for the lacking egg production of the other two birds were not clear. For sampling the eggshells were discarded, white and yolk were thoroughly mixed, separately for the eggs collected per day and hen. Subsamples from each egg-mix were radioassayed. Excreta were individually collected in intervals of 24 hours, immediately prior to each administration. From the hens the following tissues and organs were dissected and radioassayed: kidney, liver, gizzard without lining and contents, skin with subcutaneous fat, breast muscle and thigh muscle and subcutaneous fat. After recording the weights the organs were transferred into ice-cooled vessels. The tissues and organs with the exception of the subcutaneous fat were thoroughly homogenised using a meat mincer after adding some pieces of dry ice. The subsamples of the resulting tissue pulp were weighed, freeze-dried and weighed again. Subsamples were subjected to combustion followed by LSC analysis of combustion gases in the freeze-dried state because the existence of volatile components could be ruled out. In parallel subsamples were radioassayed in the wet state using the BTS-tissue solubiliser .

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

Metabolism study in the laying hen

3.3.7	Metabolite isolation and purification, quantitation and identification	The biological material taken for the quantitative analysis of metabolites was combined from all test animals. Samples of liver, muscle (composite consisting of thigh and breast), subcutaneous fat and an egg-mix were used. Egg mixture, liver, muscle and fat homogenates were separated by repetitive extraction with water, methanol and acetonitrile and partitioning with unpolar organic solvents from bulk amounts of matrix elements. All separation steps were checked by LSC. The final solutions containing the major part of the total radioactivity from the original samples were subjected to HPLC. **Analysis: Analysis and identification of metabolites:** RP-HPLC with UV-spectrophotometer and flow-through radioactivity detector. Identification of peak components using reference substances with co-chromatography. H-NMR –spectra were recorded on a 300 MHz spectrometer. Mass spectra were recorded using EI-and CI-ionisation techniques. **Measurement of radioactivity:** Measurement of solid samples using LSC: For samples of organs with weights below 500 mg or residues with a low detection limit, samples were weighed and combusted in an oxygen atmosphere using an oxidiser. Radioactivity in trapped combustion gases was measured by LSC. Fatty organs and tissues were solubilised by means of a tissue solubiliser. Radioactivity from aliquots was measured by LSC. Liquid samples were added with scintillation gel and measured by LSC.

4 RESULTS AND DISCUSSION

4.1 Absorption and excretion

The recovery of radioactivity and the excretion pattern after a triple oral administration of 10 mg per day is shown in Table A6.15.3/04-3. Until sacrifice, 50 hours after the first administration, the excretion amounted on average to about 50 % of the radioactivity administered with each dose. This indicates that the excretion rate was very high since about one half of the administered radioactivity was eliminated from the body within 24 hours after each the first and the second administration.
In line with the results from the first study it can be concluded from the high concentration in kidneys that the bulk of the radioactivity was excreted with the urinary fraction of the excreta.

An extremely low amount of 0.09 % of the total dose was found in the eggs, 24 hours after the first administration. The equivalent concentration in eggs ranged from 0.043 µg/g after 8 hours to 0.803 µg/g after 32 hours of the first dose.

At sacrifice, 2 hours after the last administration, the total residue in the organs and tissues dissected from the body was calculated and estimated to be about 8 % of the radioactivity present in the body at that time. In detail, the radioactive residues were estimated to be about 0.6 % in total skin, 0.9 % in total fat and about 4.5 % in the total musculature. These values were based on the assumption that skin, fat and musculature account for 4 %, 12 % and 40 % of the body weight, respectively.
The highest equivalent concentrations were measured in the kidneys and in the liver. These results reflect the importance of these tissues as the main excretory organs. These values were followed in decreasing order by the concentrations determined in the skin, gizzard and subcutaneous fat. These data were in agreement with the first part of this study. The resulting residue levels of the total radioactivity in the edible tissues and organs at the time of the maximum plasma concentration are given in Table A6.15.3/04-4.

4.2 Metabolism

The distribution of radioactivity in combined eggs and dissected organs used for metabolite identification are given in Table A6.15.3/04-5. The overall identification or characterisation rate of the residues averaged 67.5 % for all investigated matrices. The quantitative distribution of imidacloprid and the identified metabolites in eggs and edible tissues with their respective quantities are listed in Table A6.15.3/04-7.

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Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In a study conducted according to EPA guideline 171-4, the absorption, distribution, excretion and metabolism of imidacloprid in laying was elucidated after three consecutive daily administrations of radiolabelled test substance administered by intubation of an aqueous solution once daily at a target dose level of first 50 and finally 10 mg/kg bw. The treated animals were sacrificed at plasma peak level as determined after the first administration.

Samples of eggs and excreta were taken at pre-determined times; organs and tissues (liver, kidney, heart, muscle, gizzard, skin and subcutaneous fat) were taken after sacrifice on day 3.

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

5.2 Results and discussion

Until sacrifice, 50 hours after the first administration, the excretion amounted on average to about 50 % of the radioactivity administered with each dose. This indicates that the excretion rate was very high since about one half of the administered radioactivity was eliminated from the body within 24 hours after each the first and the second administration. In line with the results from the first study it can be concluded from the high concentration in kidneys that the bulk of the radioactivity was excreted with the urinary fraction of the excreta.

An extremely low amount of 0.09 % of the total dose was found in the eggs, 24 hours after the first administration. The equivalent concentration in eggs ranged from 0.043 µg/g after 8 hours to 0.803 µg/g after 32 hours of the first dose.

At sacrifice, 2 hours after the last administration, the total residue in the organs and tissues dissected from the body was calculated and estimated to be about 8 % of the radioactivity present in the body at that time. In detail, the radioactive residues were estimated to be about 0.6 % in total skin, 0.9 % in total fat and about 4.5 % in the total musculature. The highest equivalent concentrations were measured in the kidneys and in the liver. These results reflect the importance of these tissues as the main excretory organs. These values were followed in decreasing order by the concentrations determined in the skin, gizzard and subcutaneous fat. These data were in agreement with the first part of this study.

The overall identification or characterisation rate of the residues averaged 67.5 % for all investigated matrices. Imidacloprid olefin (M(06) was the predominant metabolite in all samples analyzed, ranging From 15.30% in liver to 28.69% in eggs. Other significant level (greater than or approaching 10%) metabolites were:
Eggs: ring opened guanidineM(11) and imidacloprid-5-hydroxy (M(01) at 17.88 and 10.05%, respectively
Liver: ring opened guanidines M(10) and M(11) at 15.94% and 8.98%, respectively
Muscle: imidacloprid-5-hydroxy (M(01) 8.61%.
Fat: Parent compound and M(01) at 12.35% and 9.68%, respectively .

Section A6.15.3/04

Annex Point IIIA, XI.1.4

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

Metabolism study in the laying hen

5.3	**Conclusion**	The metabolism of imidacloprid in poultry followed three routes of degradation as shown in Figure A6.15.3/04-1. - Hydroxylation of the imidazolidine ring which gives rise to imidacloprid-5-hydroxy and imidacloprid-4-hydroxy (M01, M02). Dehydration yields imidacloprid-olefine (M06). - Reduction and loss of the nitro group on the imidazolidine ring yields imidaclopriddihydroxyguanidine (M17). - Opening of the imidazolidine ring with loss of the ethyl group and subsequent oxidation to imidacloprid-ring-open-nitroguanidine which is transformed by loss of the nitro-group into imidacloprid-ring open guanidine (M10). This compound could also be formed from imidacloprid-dihydroxyguanidine (M17). imidacloprid-ring-open-guanidine is then hydrolysed to imidacloprid-ring-open-urea (M13) and imidacloprid-AMCP (M16) which is oxidised to 6- chloronicotinic acid (M14). Looking across both studies, it can be concluded that he metabolisation of imidacloprid in poultry is very high. Residue transfer to eggs was low. At sacrifice 2 hours after last administration, approximately 8% of total residue was found in edible organs. Parent compound, imidacloprid olefin and imidacloprid 5-hydroxy form most of the residue detected.
5.3.1	Reliability	1
5.3.2	Deficiencies	No, with the understanding the study must be coupled with A6.15.3/03.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/04/05
Materials and Methods	Acceptable.
Results and discussion	The applicant's version is acceptable.
Conclusion	See also point A6.15.3/03. The presented metabolism study in laying hen has been previously described in the imidacloprid DAR for Annex I inclusion according to Directive 91/414/EEC. Crop use related MRLs for imidacloprid in EU Member States have been defined and the proposed residue definition for risk assessment is "imidacloprid including the degradation and reaction products, determined as 6-chloronicotinic acid; calculated as imidacloprid", the residue definition for monitoring is "sum of imidacloprid and its metabolites imidacloprid-5-hydroxy and imidacloprid-olefine, expressed as imidacloprid". Measurable residues in food or feed from the use of imidacloprid in PT18 biocidal products are not expected. Therefore, an additional exposure to humans through diet arising from the use of imidacloprid as a biocide can be excluded. No MRLs specific to biocidal product uses are necessary.
Reliability	1
Acceptability	Acceptable
Remarks	None
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.15.3/04-1 Reference substances used for identification of metabolites

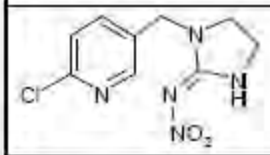
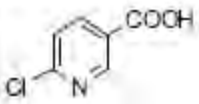
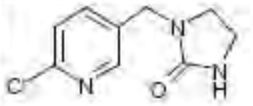
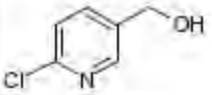
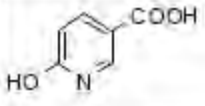
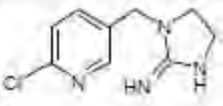
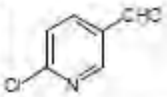
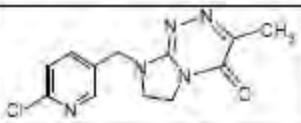
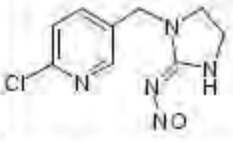
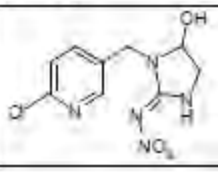
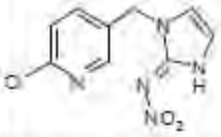
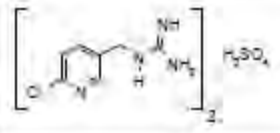
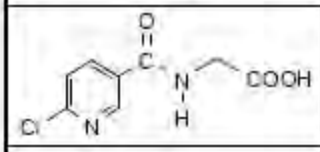
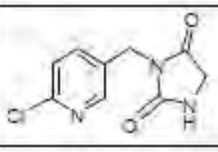
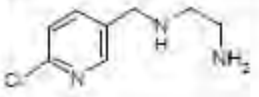
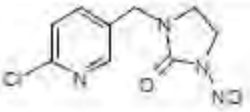
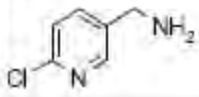
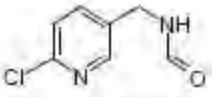
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid as		imidacloprid-6-CNA **M14**
	imidacloprid-urea **M12** (NTN33519)		imidacloprid-CHMP **M28** (DIJ9805)
	6-hydroxy-nicotinic acid **(M18)** (GBH4315)		imidacloprid-desnitro NTN38014 **M09**
	MAT 10249-0		imidacloprid-triazinone **M25**
	imidacloprid-nitrosimine **M07** (WAK3839)		imidacloprid-5-hydroxy **M01** (WAK4103)
	imidacloprid-olefine **M06** (NTN 35884)		imidacloprid-ring-open-guanidine **M10** (WAK4126)
	imidacloprid-6-CNA-glycine **M15** WAK3583		imidacloprid-5-keto-urea **M33** (DIJ10048)
	imidacloprid-PEDA **M22** DIJ 9646-2		NTN37572
	imidacloprid-AMCP **M16** GSE1478		imidacloprid-formyl-AMCP **M40** (GSE2712)

Table A6.15.3/04-1 Reference substances used for identification of metabolites, continued

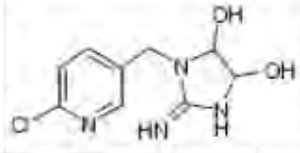
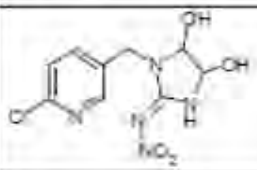
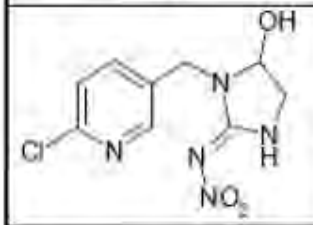
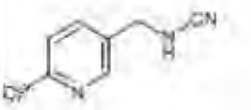
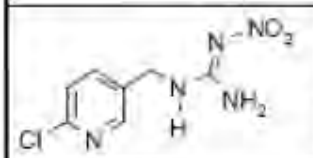
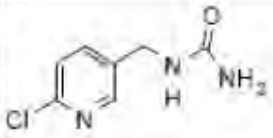
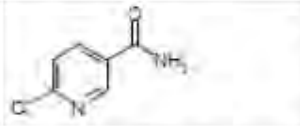
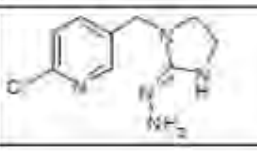
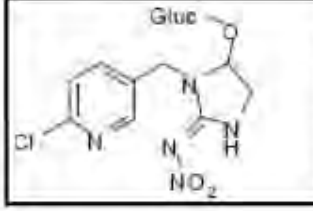
Structural formula	report names and codes	Structural formula	report names and codes
	imidacloprid-dihydroxyguanidine **M17** WAK 5031		imidacloprid-dihydroxy **M03** WAK 3772
	imidacloprid-5-hydroxy **M04** WAK 4103		imidacloprid-ring-open-guanidine **M24** WAK 4613/3
	imidacloprid-ring-open-nitroguanidine **M11** WAK 4230		imidacloprid-ring-open-urea **M13** DU 10739
	6-chloro-nicotinic amide BNF 5518 B		imidacloprid-amino-guanidine **M08** WAK 3877/4
	imidacloprid-5-hydroxy-glucoronide **M04** WAK 4103-Glucuronide		

Table A6.15.3/04-2 Recovery of radioactivity after repeated oral administration of 10 mg/kg per day to laying hens

Sample	Time after the 1st application hours	Mean values in percent of the radioactivity present in the animals (arithmetic mean ± standard deviation)
Excreta	24 48 50	51.4 ± 14.9* 47.0 ± 12.1** 5.5 ± 1.7***
Eggs	24 48	0.087* 0.184**
Calculated residues in tissues		7.81 ± 1.09***

Notes:

*: % of radioactivity administered with the 1st dose

**: % of radioactivity administered with the 1st, 2nd and 3rd dose including the non-extracted radioactivity from the first dose

***: % of totally administered radioactivity in the body at the time of sacrifice

Table A6.15.3/04-3 Equivalent concentration in the tissues and organs of laying hens at the time of sacrifice, 50 hours after the first administration

Tissue/organ	Equivalent concentration Mean ± SDVE* [µg/g]
Liver	12.75 ± 17.6
Kidney	18.88 ± 18.5
Gizzard	2.356 ± 34.8
Muscle, thigh	2.303 ± 12.8
Muscle, chest	2.102 ± 13.1
Muscle, comp.	2.203 ± 11.8
Fat, subcutaneous	1.506 ± 40.3
Skin without fat	2.931 ± 8.13

* Arithmetic mean ± coefficient of variance (%)

Table A6.15.3/04-4 Distribution of radioactivity in combined eggs and dissected organs

Tissue/organ	[µg parent/g]
Egg	0.49
Liver	12.51
Composite muscle	2.20
Composite fat	1.55

Table A6.15.3/03-5 Metabolism of [pyridinyl-14C-methylene]-imidacloprid in eggs, organs, tissues of laying hens 50 hours after repeated oral administration of 3 x 10 mg as/kg bw

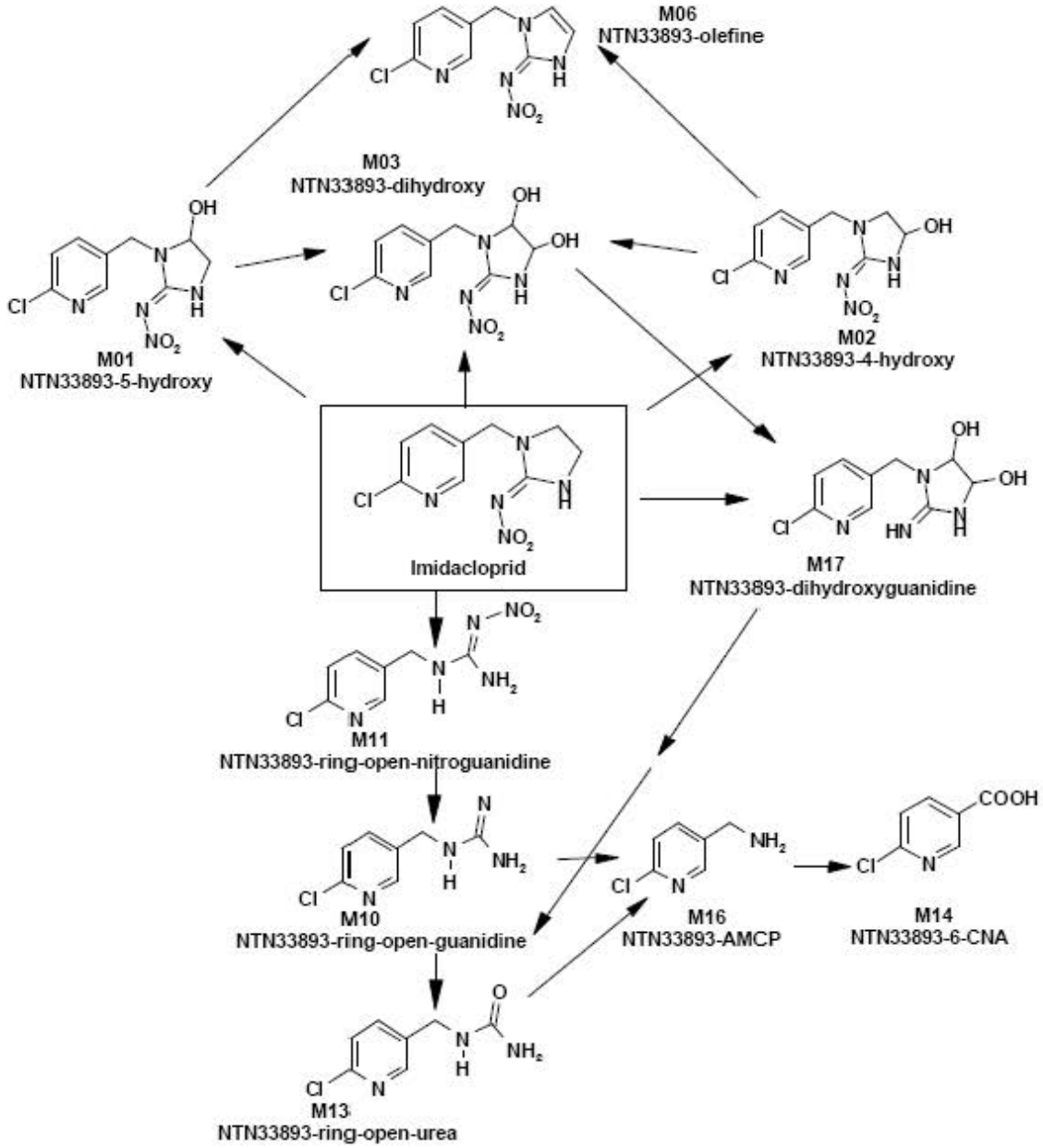
Parent compound/	Eggs		Liver		Muscle		Fat	
metabolite	[µg/g]	%	[µg/g]	%	[µg/g]	%	[µg/g]	%
imidacloprid	0.023	4.83	-	-	0.138	6.26	0.191	12.35
imidacloprid-5-hydroxy (M01)	0.049	10.05			0.190	8.61	0.150	9.68
imidacloprid-4-hydroxy (M02)	0.028	5.70	1.914*	8.51*	0.102	4.62	0.036	2.34
imidacloprid-dihydroxy (M03)	0.002	0.47			-	-	-	-
imidacloprid-olefine (M06)	0.140	28.69	1.123	15.30	0.589	26.74	0.350	22.55
**-ring-open-guanidine (M10)	0.019	3.96	1.994	15.94	0.136	6.16	0.065	4.22
**-ring-open-nitroguanidine(M11)	0.087	17.88	1.065	8.98	0.148	-	0.079	5.11
imidacloprid-ring-open-urea(M13)	0.009	1.81	0.970	7.75	0.081	3.67	0.021	1.38
imidacloprid-6-CNA (M14)	-	-	0.309	2.47	-	-	0.029	1.86
imidacloprid-AMCP (M16)	0.019	3.90	0.244	1.95	0.079	3.60	0.023	1.49
**-dihydroxyguanidine (M17)	0.004	0.82	0.274	2.19	0.030	1.36	-	-
Sum identified	0.380	78.11	7.893	63.09	1.493	67.73	0.944	60.98

Notes:

* = sum of imidacloprid-5-hydroxy, imidacloprid-4-hydroxy and imidacloprid-dihydroxy

**= imidacloprid is part of the full report name

Figure A6.15.3/04-1: Proposed pathway of imidacloprid in laying hen



Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to rainbow trout (salmo gairdneri)*

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2.1, II A, 8.2.1 /02*
	Authors (year)	[redacted] (1988b)
	Title	The acute toxicity of NTN 33893 techn. to rainbow trout (*salmo gairdneri*) in a static test
	Company, report No.	Bayer CropScience AG, Report-No.: FF-210 M-006827-01-2
	Date	1988-03-03
	Testing facility	[redacted]
	Dates of work	October 5, 1987 – October 9, 1987
	Test substance(s)	Molecule(s): imidacloprid Substance(s): NTN 33893 Z (Batch-No.: 180587)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	EEC Directive 79/831, Annex V, OECD 203
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893 tech., purity: 95.3 %, specification: (charge : 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	Further relevant properites	none
3.1.5	Method of analysis	HPLC with UV detection, RA-696/October 2, 1987
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble
3.3	**Reference substance**	no

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to rainbow trout (salmo gairdneri)*

3.4	**Testing procedure**		
3.4.1	Dilution water	Young rainbow trout (mean body length 5.3 cm, mean body weight 1.3 g, see Table A7.4.1.1/01-1): 10 fish per test concentration were exposed for 96 hours under static conditions to nominal concentrations of 0 (control), 50, 89, 158, 281 and 500 mg as/L. All calculations refer to nominal values, which were analytically confirmed (measured > 80 % of nominal concentrations).	x
3.4.2	Test organisms		
3.4.3	Test system		
3.4.4	Test conditions		x
3.4.5	Duration of the test		
3.4.6	Test parameter	Study conducted in accordance with OECD 203, no water, test system, conditions or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	Yes, at 0, 24, 96 h	
3.4.9	Statistical analysis	LC50 values with 95% confidence intervals were calculated for each 24 hour period according to the method of Thompson and Weil.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal: 0 (control), 50, 89, 158, 281 and 500 mg as/L Confirmed	
4.2.2	Actual concentrations of test substance	T0: 0, 53.4, 98.9, 176, 304, 533 T24: 51.5, 91.4, 167, 298, 400* T96: 51.8, 100.5, 174, 328, highest concentration not measured due to 100 mortality by 24 h * a.s. drop due to limit of solubility, sediment observed on bottom of tank	x
4.2.3	Effect data (Mortality)	See Table A7.4.1.1/01-2 and 3. Mortality was observed in the two top concentrations (281 and 500 mg as/L).	
4.2.4	Concentration/response curve	Concentration-activity ratio is narrow; 0 and 100% mortality occur in neighboring concentrations at a factor of progression of 1.8	
4.2.5	Other effects	Symptoms of intoxication occured mainly at 158 mg as/L and higher concentrations. The toxic symptoms were noted as: swimming behaviour slightly irregular (light symptom), apathetic, lying on side/ back and staggering.	
4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	none	
4.3.2	Nature of adverse	none	

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to rainbow trout (salmo gairdneri)*

	effects	
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute fish toxicity study conducted according to EEC Directive 79/831, Annex V, OECD 203, young rainbow trout *(salmo gairdneri)* 10 fish per test concentration were exposed for 96 hours under static conditions to nominal concentrations of 50, 89, 158, 281 and 500 mg as/L. All calculations refer to nominal values, which were analytically confirmed (measured > 80 % of nominal concentrations). Mortality and symptoms of toxicity were reported.
5.2	**Results and discussion**	Mortality was observed in the two top concentrations (281 and 500 mg as/L). Symptoms of intoxication occured mainly at 158 mg as/L and higher concentrations. The toxic symptoms were noted as: swimming behaviour slightly irregular (light symptom), apathetic, lying on side/ back and staggering.
5.2.1	LC_0	96 hours : 158mg/l (by observation of no mortality)
5.2.2	LC_{50}	96 hours : 211 mg/l (151-281 mg a.s./l 95% C.I.)
5.2.3	LC_{100}	96 hours : 281 mg/l (by observation of 100% mortality)
5.3	**Conclusion**	In a 96 hours static acute toxicity study meeting the validity criteria with *Oncorhynchus mykiss* (former *Salmo gairdneri*) the LC50 of imidacloprid (technical active substance) was determined to be 211 mg as/L (nominal). The NOEC was 89 mg as/L (nominal). The nominal concentrations were analytically confirmed.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.1/01 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to rainbow trout (salmo gairdneri)*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/07
Materials and Methods	Applicant's version is acceptable with the following comments/additions: 3.4.1: Dilution water: Reconstituted water (Ca^{2+}: 2 mmole/l; Mg^{2+}: 0.5 mmole/l; Na^{+}: 0.77mmole/l; K^{+}: 0.077 mmole/l; HCO_3^-: 0.77 mmole/l; Cl^-: 4.077 mmole/l; SO_4^{2-}: 0.5 mmole/l) 3.4.4: Test conditions: 16:8 hours light/dark;
Results and discussion	Applicant's version is acceptable with the following comments/additions: 4.2.2: Test substance lying at the bottom was observed in all test aquaria. At the highest test concentration of 500 mg/l after 3 hours also heavy turbidity caused by the test substance was observed. However, only in the highest test concentration the concentration dropped below 80 % of the nominal concentration.
Conclusion	Applicants's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.1/01-1: Test organisms

Criteria	Details
Species/strain	*salmo gairdneri*
Source	Forellenzucht Linn, D-5940 Lennestadt, FRG
Wild caught	No
Age/size	Mean body length 5.3 cm, mean body weight 1.3 g
Kind of food	Tetramine, Tetra-Werke
Amount of food	Not specified, according to guideline
Feeding frequency	Not specified, according to guideline
Pretreatment	14 days in test water and at test temperature
Feeding of animals during test	Not after 48 hours before start or during the test

Table A7.4.1.1/01-2: Mortality data

Test-Substance Concentration nominal [mg/l]	Mortality/Symptoms/No. fish							
	Number				Percentage Mortality			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0/0/10	0/0/10	0/0/10	0/0/10	0	0	0	0
50	0/0/10	0/0/10	0/0/10	0/0/10	0	0	0	0
89	0/0/10	0/10/10	0/0/10	0/0/10	0	0	0	0
158	0/10/10	0/10/10	0/10/10	0/10/10	0	0	0	0
281	6/10/10	10/10/10	--	--	60	100	100	100
500	10/10/10	--	--	--	100	100	100	100
LC50	265	211	211	211				
LC50 range	220-320	158-281	158-281	158-281				
Temperature [°C]	15.7	15.1	15.2	14.8				
pH	8.0-8.1							
Oxygen [mg/l]	9.9-10.8 (92-101% saturation)							

Table A7.4.1.1/01-3 : Acute toxicity of imidacloprid to Rainbow trout

Test substance	Tech. as
Test object	Rainbow trout
Exposure	96 h, static
LC_{50} [mg as/L]	211
Lowest observed effect concentration (LOEC) [mg as/L]	158
No observed effect concentration (NOEC) [mg as/L]	89*

* Transient irregular swimming behaviour at 48 h (not relevant)

Table A7.4.1.1/01-4: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Section A7.4.1.1/02 Acute toxicity to fish

Section A7.4.1.1/03 *Acute toxicity to rainbow trout(Oncorhynchus mykiss)*

Annex Point IIA7.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: B.9.2.1, II A, 8.2.1 /04*	
	Authors (year)	[redacted] (1990b)	
	Title	Acute toxicity of NTN 33893 to rainbow trout (*Oncorhynchus mykiss*)	
	Company, report No.	Bayer CropScience AG, Report-No.: 100349 BES Ref. : M-007019-01-1	
	Date	1990-12-12	
	Testing facility	[redacted]	
	Dates of work	April 19, 1990 – April 23, 1990	
	Authors (year)	Bussard, J. (1990)	
	Title	Method validation for the analysis of NTN-33893 in aquatic test water	
	Company, report No.	[redacted] report no. 37859 BES Ref.: M-015716-01-1	
	Date	1990-03-13	
	Testing facility	Analytical Bio-Chemistry Laboratories, Inc.	
	Dates of work	Not given	
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 9030211, 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**	U.S.-EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-1	x
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	NTN 33893 tech., purity: 95.0 %, Batch No. 903021	
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	Further relevant properites	none	

Section A7.4.1.1/02 Acute toxicity to fish

Section A7.4.1.1/03 *Acute toxicity to rainbow trout(Oncorhynchus mykiss)*

Annex Point IIA7.1

3.1.5	Method of analysis	HPLC with UV detection, validated by ABC laboratories in ABC report 37859	
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	
3.3	**Reference substance**	no	
3.4	**Testing procedure**		
3.4.1	Dilution water	Young Rainbow trout (mean body length 4.4 cm, mean body weight 1.07 g, see Table A7.4.1.1/02 & /03-1): 10 fish per test concentration were exposed for 96 hours under static conditions to nominal concentrations (corrected for 95 % purity of the test compound) of 16, 27, 45, 75 and 125 mg as/L (measured concentrations were 15, 27, 42, 64 and 83 mg as/L). All results refer to measured concentrations.Fish were monitored for mortality and behavioral changes.	x
3.4.2	Test organisms		x
3.4.3	Test system		x
3.4.4	Test conditions		x
3.4.5	Duration of the test		
3.4.6	Test parameter		
3.4.7	Sampling	Study conducted in accordance with U.S.-EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-1, no water, test system, conditions or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.8	Monitoring of TS concentration	Yes, 0 and 96h	
3.4.9	Statistical analysis	Since no mortality occurred, an LC50 and confidence limits could not be calculated	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	16, 27, 45, 75 and 125 mg as/L	
4.2.2	Actual concentrations of test substance	T0: 15, 27, 41, 57 and 61 mg as/L T96: 15, 26, 43, 71 and 105 mg as/L	
4.2.3	Effect data (Mortality)	See Table A7.4.1.1/02 & /03-2 and 3. No mortalities were recorded up to the highest test concentration. The 24, 48, 72 and 96 hour LC50-values for rainbow trout were all > 83 mg as/L. The LC50 was shown to be greater than the water solubility limits of the test material. Surface film and precipitate were (partly transiently) noted in the 42, 64 and 83 mg as/L test solutions.	
4.2.4	Concentration/resp	No mortality	

	onse curve	
4.2.5	Other effects	The 96-hour NOEC was estimated to be 42 mg as/L, based on the lack of mortality and sublethal effects. The abnormal effects of dark discoloration, fish on the bottom of the test chamber, erratic swimming and/or quiescence were observed in the 64 and 83 mg as/L test concentrations during the 96-hour exposure period.
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 fish toxicity study conducted according to EPA Guideline 72-1, young rainbow trout (*Oncorhynchus mykiss* mean body length 4.4 cm, mean body weight 1.07 g): 10 fish per test concentration were exposed for 96 hours under static conditions to nominal concentrations (corrected for 95 % purity of the test compound) of 16, 27, 45, 75 and 125 mg as/L (mean measured concentrations were 15, 27, 42, 64 and 83 mg as/L). All results refer to measured concentrations. Mortality and symptoms of toxicity were reported.
5.2	**Results and discussion**	No mortalities were recorded up to the highest test concentration. The 24, 48, 72 and 96 hour LC50-values for rainbow trout were all > 83 mg as/L. The LC50 was shown to be greater than the water solubility limits of the test material. Surface film and precipitate were (partly transiently) noted in the 42, 64 and 83 mg as/L test solutions. The 96-hour NOEC was estimated to be 42 mg as/L, based on the lack of mortality and sublethal effects. The abnormal effects of dark discoloration, fish on the bottom of the test chamber, erratic swimming and/or quiescence were observed in the 64 and 83 mg as/L test concentrations during the 96-hour exposure period.
5.2.1	LC_0	> 83 mg/l
5.2.2	LC_{50}	>83 mg/l
5.2.3	LC_{100}	>83 mg/l
5.3	**Conclusion**	In a 96 hours static acute toxicity study in agreement with *Oncorhynchus mykiss* the LC50 of imidacloprid (technical active substance) was determined to be > 83 mg as/L (measured). The NOEC for behavioural effects was 42 mg as/L (measured).
5.3.1	Reliability	1 Although the oxygen content fell below 60 % during the study, the same tendency occurred in treatment and control groups, and no effects (mortality and observations) observed in control at any time. The RMS

Section A7.4.1.1/02 Acute toxicity to fish

Section A7.4.1.1/03 *Acute toxicity to rainbow trout(Oncorhynchus mykiss)*

Annex Point IIA7.1

		of the December 2005 91/414 DAR considered the study valid and used it as the key acute fish study for risk assessment.
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	2006/11/09
Materials and Methods	Applicant's version is acceptable with the following comments/additions: 2.1: The test was conducted following the procedures outlined in ABC Protocol No. 7601-SEP (revised November 16, 1989). The protocol was patterned after the methods described in "Methods for acute toxicity tests with fish, macroinvertebrates and amphinbians" and "Standard methods for the examination of water and wastewater". 3.4.1: Dilution water: soft blended water, 40-48 mg/l CaCO3; O2: 9.1 mg/l, pH: 7.9 3.4.2: In table A7.4.1.1/02-1 the size of the test organisms is erroneously given with 44 cm instead of 4.4 cm. 3.4.3: Test system: To increase dispersion of the test compound in the dilution water, 1.5 ml DMF was added to each sample weight (DMF concentration 0.1 ml/l). 3.4.4: Test conditions: O_2 content decreased from 9.1 – 9.3 mg/l at 0 h to 5.8 – 6.8 mg/l at 48 h and to 5.6 -6.5 mg/l at 96 h. That means that during the exposure period, the oxygen content was partly < 60 % of saturation.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

Section A7.4.1.1/02 Acute toxicity to fish

Section A7.4.1.1/03 *Acute toxicity to rainbow trout(Oncorhynchus mykiss)*

Annex Point IIA7.1

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

Table A7.4.1.1/02 & /03-1: Test organisms

Criteria	Details
Species/strain	*Oncorhynchus mykiss*
Source	Mt. Lassen trout farms, Red bluff, California
Wild caught	No
Age/size	Mean body length 44 cm, mean body weight 1.07 g
Kind of food	Brine shrimp or fish food from Zeigler Bros., Inc.
Amount of food	Not specified, according to guideline
Feeding frequency	daily
Pretreatment	4 wk acclimation period in tanks with recirculated water, 2 days in temperature acclimation tank
Feeding of animals during test	Not after 48 hours before start or during the test

Table A7.4.1.1/02 & /03-2: Symptoms data

Test-Substance Concentration measured [mg/l]	Symptoms							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0/10	0/10	0/10	0/10	0	0	0	0
Solvent control	0/10	0/10	0/10	0/10	0	0	0	0
15	0/10	0/10	0/10	0/10	0	0	0	0
27	0/10	0/10	0/10	0/10	0	0	0	0
42	0/10	0/10	0/10	0/10	0	0	0	0
64	10/10	10/10	10/10	10/10	100	100	100	100
83	10/10	10/10	10/10	10/10	100	100	100	100
Temperature [°C]	13							
pH	7.1-7.9							
Oxygen [mg/l]	5.7-9.3 for 42 and 83, 5.7-9.2 for 15, 5.6-9.1 for control							

Table A7.4.1.1/02 & /03-3 : Acute toxicity of imidacloprid to Rainbow trout

Test substance	Tech. as
Test object	Rainbow trout
Exposure	96 h, static
LC_{50} [mg as/L]	> 83
Lowest observed effect concentration (LOEC) [mg as/L]	64
No observed effect concentration (NOEC) [mg as/L]	42

Table A7.4.1.1/02 & /03-4: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fulfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation		X*
Concentration of test substance ≥80% of initial concentration during test	X	

*however, does not invalidate study results as control was unaffected under same circumstances

Section A7.4.1.1/04 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to golden orfe (Leuciscus idus melanotus)*

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2.1, II A, 8.2.1 /01*
	Authors (year)	[redacted] (1987)
	Title	The acute toxicity of NTN 33893 techn. to golden orfe (*Leuciscus idus melanotus*) in a static test
	Company, report No.	Bayer CropScience AG, Report-No.: FO-1042 BES Ref. : M-006830-01-2
	Date	1987-10-26
	Testing facility	[redacted]
	Dates of work	October 05, 1987 – October 09, 1987
	Test substance(s)	Molecule(s): imidacloprid Substance(s): NTN 33893 Z (Batch-No.: 180587)
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience AG
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	according to EEC-guideline "EEC DIRECTIVE 79/831/WG, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1. Acute Toxicity for Fish“
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 tech., purity: 95.3 %, Batch No. 18-587
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	Further relevant properites	none
3.1.5	Method of analysis	HPLC with UV detection, RA-696/October 2, 1987
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble
3.3	**Reference**	no

Section A7.4.1.1/04 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to golden orfe (Leuciscus idus melanotus)*

	substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	The acute toxicity of NTN 33893 techn. to Golden Orfe was determined in a 96-h-static test The nominal concentrations tested were 100, 178, 316, 562 and 1000 mg as/L (nominal) and a control without any additions.	x
3.4.2	Test organisms		
3.4.3	Test system		
3.4.4	Test conditions	Study conducted in accordance with EEC-guideline "EEC DIRECTIVE 79/831/WG, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1, no water, test system, conditions or sampling deviations noted by the RMS of the December 2005 91/414 DAR	x
3.4.5	Duration of the test		
3.4.6	Test parameter	See Table A7.4.1.1/04-1.	
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	Yes, at 0, 24, 96 h (at levels with surviving fish)	
3.4.9	Statistical analysis	LC50 values with 95% confidence intervals were calculated for each 24 hour period according to the method of Thompson and Weil.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	100, 178, 316, 562 and 1000 mg as/L	
4.2.2	Actual concentrations of test substance	T0: 112, 208, 357, 645 and 1002 mg as/L T24: 106, 199, 341, 483 and 547 mg as/L T96: 106, 193 (only doses with live fish)	x
4.2.3	Effect data (Mortality)	See Table A7.4.1.1/04-2. All calculations refer to nominal values, because analytical control of the concentrations showed that the measured values were greater than 85 % of the nominal values in all aquaria with the exception of the highest concentration, where only 54 % was found after 24 hours. This, however, had no influence on the results of the test, because, as in this concentration, in the two next lower concentrations (316 and 562 mg/L) all fish died within 24 hours and the concentrations of the test substance remained constant. The 96-hour LC50 of the technical active substance was determined to be 237 mg as/L with a range from 178 to 316 mg as/L. The lowest lethal concentration was 316 mg as/L	
4.2.4	Concentration/response curve	Concentration-activity ratio is narrow; 0 and 100% mortality occur in neighboring concentrations at a factor of progression of 1.778	
4.2.5	Other effects	Symptoms of intoxication were not observed in surviving fish.	

Section A7.4.1.1/04 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to golden orfe (Leuciscus idus melanotus)*

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 fish toxicity study conducted according to EEC directive, 1984, the acute toxicity of NTN 33893 techn. to Golden Orfe was determined in a 96-h-static test The nominal concentrations tested were 100, 178, 316, 562 and 1000 mg as/L (nominal) and a control without any additions.
5.2	**Results and discussion**	At 316 mg/l and higher, all fish died. The lowest lethal concentration was 316 mg as/L, and the no-observed-effect-concentration (NOEC) 178 mg as/L. No symptoms were observed in surviving fish. The 96-hour LC50 of the technical active substance was determined to be 237 mg as/L with a range from 178 to 316 mg as/L. This range is derived from the two adjacent concentrations with a spacing factor of 1.778, in which 0 and 100 % mortality were observed.
5.2.1	LC_0	$\geq$ 178 mg/l
5.2.2	LC_{50}	237 mg/l
5.2.3	LC_{100}	$\leq$ 316 mg/l
5.3	**Conclusion**	In a 96 hours static acute toxicity study meeting the validity criteria with *Leuciscus idus melanotus* the LC50 of imidacloprid (technical active substance) was determined to be 237 mg as/L. The NOEC was 178 mg as/L.
5.3.1	Reliability	1
5.3.2	Deficiencies	None noted by the RMS of the December 2005 91/414 DAR

Section A7.4.1.1/04 Acute toxicity to fish

Annex Point IIA7.1 *Acute toxicity to golden orfe (Leuciscus idus melanotus)*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/07
Materials and Methods	Applicant's version is acceptable with the following comments/additions: 3.4.1: Dilution water: Reconstituted water (Ca^{2+}: 2 mmole/l; Mg^{2+}: 0.5 mmole/l; Na^{+}: 0.77mmole/l; K^{+}: 0.077 mmole/l; HCO_3^-: 0.77 mmole/l; Cl^-: 4.077 mmole/l; SO_4^{2-}: 0.5 mmole/l) 3.4.4: Test conditions: 16:8 hours light/dark
Results and discussion	Applicant's version is acceptable with the following comments/additions: 4.2.2: Test substance lying at the bottom was observed in all test aquaria. At the 2 highest test concentrations (562 and 1000 mg/l) after 2-4 hours also heavy turbidity caused by the test substance was observed. However, only in these 2 highest test concentrations the concentration dropped below 80 % of the nominal concentration.
Conclusion	Applicants's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.1/04-1: Test organisms

Criteria	Details
Species/strain	*Leucuscus idus melanotus*
Source	Fischzucht Eggers, Hohenwestedt, FRG
Wild caught	No
Age/size	Mean body length 6.4 cm, mean body weight 2.4 g
Kind of food	Tetramine, Tetra-Werke
Amount of food	Not specified, according to guideline
Feeding frequency	Not specified, according to guideline
Pretreatment	14 days in test water and at test temperature
Feeding of animals during test	Not after 48 hours before start or during the test

Table A7.4.1.1/04-2: Mortality data

Test-Substance Concentration nominal [mg/l]	Mortality/Symptoms/No. fish							
	Number				Percentage Mortality			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
0	0/0/10	0/0/10	0/0/10	0/0/10	0	0	0	0
100	0/0/10	0/0/10	0/0/10	0/0/10	0	0	0	0
178	0/0/10	0/0/10	0/0/10	0/0/10	0	0	0	0
316	10/10/10	--	--	--	100	100	100	100
562	10/10/10	--	--	--	100	100	100	100
1000	10/10/10	--	--	--	100	100	100	100
LC50	237	237	237	237				
LC50 range	178-316	178-316	178-316	178-316				
Temperature [°C]	21.7	21.0	20.7	20.0				
pH	8.1							
Oxygen [mg/l]	8.2-9.3 (92-101% saturation)							

Table A7.4.1.1/04-3: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	X	
Concentration of dissolved oxygen in all test vessels > 60% saturation	X	
Concentration of test substance ≥80% of initial concentration during test	X	

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: B.9.2.3, II A, 8.2.4 /01*	
	Authors (year)	Young, B. M.; Hicks, S. L. (1990)	
	Title	Acute toxicity of NTN 33893 to *Daphnia magna*	
	Company, report No.	Bayer CropScience AG, Report-No.: 100245 BES Ref. : M-006821-01-1	
	Date	1990-09-12	
	Testing facility	[redacted]	
	Dates of work	June 13, 1990 – June 15, 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**	EPA-FIFRA § 72-2, OECD 202	x
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	NTN 33893, purity: 95.4 %, Specification: (Lot No.: 9030211); 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	Further relevant properites	none	
3.1.5	Method of analysis	HPLC with UV detection, validated by ABC laboratories in ABC report 37859	
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	
3.3	**Reference substance**	no	
3.4	**Testing procedure**		
3.4.1	Dilution water	Instars of *Daphnia magna* (< 24 h old, see Table 7.4.1.2/01-1) in a static	

3.4.2	Test organisms	test system were exposed for 48 h to nominal concentrations of 16, 27, 45, 75 and 125 mg a.s./L. Mean measured concentrations were 15, 25, 42, 71 and 113 mg as/L (20 animals per test concentration). Immobilization and behavior were monitored over 48 hours. Four, 24 and 48 hr EC50 values were determined.	x
3.4.3	Test system		
3.4.4	Test conditions		
3.4.5	Duration of the test		
3.4.6	Test parameter	Study conducted according to EPA-FIFRA § 72-2, OECD 202 guidelines, no water, test system, conditions or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	Yes @ 0 and 48 h	
3.4.9	Statistical analysis	EC50 by probit, dose response slope by linear regression	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations of 16, 27, 45, 75 and 125 mg a.s./L	
4.2.2	Actual concentrations of test substance	T0: 15, 25, 43, 71, 116 mg a.s./L T48: 15, 24, 41, 71, 109 mg a.s./L Mean measured concentrations were 15, 25, 42, 71 and 113 mg as/L	
4.2.3	Effect data (Immobilisation)	See Table A7.4.1.2/01-2 for effects. The EC50-value for *Daphnia magna* was calculated to amount to 85 mg as/L (71-113 mg/L, 95% C.I.). Based on the absence of immobility and other abnormal effects, the NOEC observed for imidacloprid was 42 mg as/L after 48 hours (See Table A7.4.1.2/01-3).	
4.2.4	Concentration / response curve		
4.2.5	Other effects	The calculated 48-hours dose response slope was 11.	
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 toxicity study conducted according to EPA-FIFRA § 72-2, OECD 202 guidelines, instars of *Daphnia magna* (< 24 h old) in a static test system were exposed for 48 h to nominal concentrations of 16, 27, 45, 75 and 125 mg imidacloprid./L. Mean measured concentrations were 15, 25, 42, 71 and 113 mg as/L (20 animals per test concentration). Immobilization and behavior were monitored over 48 hours.	
5.2	**Results and**	The EC50-value for *Daphnia magna* was calculated to amount to 85 mg	

	discussion	as/L (71-113 mg/L, 95% C.I.). Based on the absence of immobility and other abnormal effects, the NOEC observed for imidacloprid was 42 mg as/L after 48 hours . The calculated 48-hours dose response slope was 11.
5.2.1	EC_0	$\geq$ 42 mg a.s./L
5.2.2	EC_{50}	85 mg a.s./L (71-113 mg/L, 95% C.I.).
5.2.3	EC_{100}	$\leq$ 113 mg a.s./L
5.3	**Conclusion**	In a 48 hours static acute toxicity study meeting the validity criteria with *Daphnia magna* the EC50 of imidacloprid (NTN 33893) was determined to be 85 mg as/L. The NOEC was 42 mg as/L (mean measured concentrations).
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	2006/11/08
Materials and Methods	Applicant's version is acceptable with the following comments/additions: 2.1: No guideline is given in the study report, but it is stated that the test was performed using methods outlined in ABC Protocol 7806-SEP. According to the description however the method is in accordance to OECD 202. 3.4.4: 2 replicates per concentration with 10 daphnids per replicate
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.2/01-1: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	ABC Laboratories in house culture
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	At least every 3 days
Feeding of animals during test	No

Table A7.4.1.2/01-2: Immobilisation data

Test-Substance Concentration (effective) [mg/l]	Immobile *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Tempera-ture [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0/20	0/20	0	0	8.3	8.3	20
15 mg a.s./l	0/20	0/20	0	0	8.4	8.3	20
25 mg a.s./L	0/20	0/20	0	0	8.2	8.3	20
42 mg a.s./L	0/20	0/20	0	0	8.1	8.3	20
71 mg a.s./L	0/20	2/20	0	10	8.2	8.3	20
113 mg a.s./L	0/20	10/10*	0	100	8.3	8.4	20
* daphnids coated with extraneous material on antennae							

Table A7.4.1.2/01-3: Toxicity of imidacloprid to waterfleas

Test substance	Tech. as
Test object	*Daphnia magna*
Exposure	48 h, static
EC_{50} [mg as/L]	85
Lowest observed effect concentration (LOEC) [mg as/L]	71
No observed effect concentration (NOEC) [mg as/L]	42

Table A7.4.1.2/01-4: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to larvae of chironomus riparius*

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1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2.6, II A, 8.2.7/06*
	Authors (year)	Dorgerloh, M.; Sommer, H. (2002a)
	Title	Acute toxicity of imidacloprid (tech.) to larvae of Chironomus riparius
	Company, report No.	Bayer CropScience AG, Report-No.: DOM 22031 BES Ref. : M-058794-01-1
	Date	2002-04-12
	Testing facility	[redacted]
	Dates of work	Febuary 28, 2002 to March 12, 2002
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid (Batch-No.: M03872)
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**	none (method essentially equivalent to OECD Guideline 202 from April 4, 1984)
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	Imidacloprid active substance-content: 99.9 % (Batch-No.: M03872), Development-No.: 3000125426
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	Further relevant properites	none
3.1.5	Method of analysis	HPLC with UV detection, RA 454/90, June 8, 1990
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble
3.3	**Reference substance**	No

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to larvae of chironomus riparius*

3.4	**Testing procedure**		
3.4.1	Dilution water	See Tables A.7.4.1/02-1 to 3. Parameters in line with requirements of OECD 202 of April, 1984.	x
3.4.2	Test organisms		
3.4.3	Test system	Larvae of *Chironomus riparius* (1st instars < 2-3 days old, 3 beakers per test concentration and 6 beakers as control with 10 animals each) were exposed for 24 hours in a static test system to aqueous concentrations of 8, 16, 32, 64 and 128 µg as/L (nominal initial). Measured concentrations of the test substance were 95.6 to 101.9 % (on average 99.4 %) of nominal on day 0.	
3.4.4	Test conditions		
3.4.5	Duration of the test		
3.4.6	Test parameter		
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	Yes on day 0	
3.4.9	Statistics	Probit analysis after the maximum likelihood method	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal water concentration 8, 16, 32, 64 and 128 µg as/l	
4.2.2	Actual concentrations of test substance	Mean measured concentrations were 8.05, 16.3, 30.6, 62.9 and 129 µg as/l	x
4.2.3	Effect data (Immobilisation)	See Table A7.4.1.2/02-4 for effects. At the test level of 64 µg as/L all surviving larvae lay inactive on the bottom of the beakers.	
4.2.4	Concentration / response curve	See Table A7.4.1.2/02-5 for the LC50 value	
4.2.5	Other effects	Slope of the line of regression after Litchfield and Wilcox was s=1.33.	
4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	3.3%	
4.3.2	Nature of adverse effects	mortality	
4.4	**Test with reference substance**	Not performed	

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to larvae of chironomus riparius*

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In an acute toxicity study essentially equivalent to OECD Guideline 202 from April 4, 1984, larvae of *Chironomus riparius* (1st instars < 2-3 days old, 3 glass beakers per test concentration and 6 glass beakers as control with 10 animals each) were exposed for 24 hours in a static test system to aqueous concentrations of 8, 16, 32, 64 and 128 µg as/L (nominal initial). Measured concentrations of the test substance were 95.6 to 101.9 % (on average 99.4 %) of nominal on day 0. Mortality and symptoms were reported after 24 hours.
5.2	**Results and discussion**	At the test level of 64 µg as/L all surviving larvae laid inactive on the bottom of the beakers. The LC50 of imidacloprid (NTN 33893) was determined to be 0.0552 mg as/L (nominal, analytically confirmed): 0.0481- 0.0633 95% C.I..
5.2.1	EC_0	≥ 0.016 mg a.s./L
5.2.2	EC_{50}	0.055 mg a.s./L
5.2.3	EC_{100}	> 0.064, < 0.128 mg a.s./L
5.3	**Conclusion**	In a 24 hours static toxicity test with *Chironomus riparius,* the LC50 of imidacloprid (NTN 33893) was determined to be 0.055 mg as/L (nominal, analytically confirmed).
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	2006/11/08
Materials and Methods	Applicant's version is acceptable with the following additions/comments: 3.4.1: dilution water: M7 medium
Results and discussion	Applicant's version is acceptable with the following additions/comments: 4.2.2: Measured concentrations on day 0 (mean of 2 analyses each).
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-

Section A7.4.1.2/02 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to larvae of chironomus riparius*

	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 A7.4.1.2/02-1: 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	On day 0 a suspension with commercial ornamental fish food was added to each test container (about 12.5 mg/L)
Feeding frequency	One time at start of study
Pre-treatment	None detailed
Feeding during test	On day 0 a suspension with commercial ornamental fish food was added to each test container

Table A7.4.1.2/02-2: Test system

Criteria	Details
Renewal of test solution	no
Volume of test vessels	250 ml filled to 200 ml
Volume/animal	25/20 ml
Number of animals/vessel	10
Number of vessels/ concentration	3 for test substance, 6 for control
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/02-3: Test conditions

Criteria	Details
Test temperature	20 ± 2 C
Dissolved oxygen	7.9 mg/l
pH	8.0
Adjustment of pH	No
Aeration of dilution water	Yes
Quality/Intensity of irradiation	1002 lux
Photoperiod	16 hour daily
Total hardness	213.6 mg/l CaCO3
Alkalinity	53.4 mg/l CaCO3
Conductivity	588 µS/cm

Table A7.4.1.2/02-4: Toxicity of imidacloprid to larvae of *Chironomus riparius*

Test-Substance Concentration (nominal) [µg /l]	Number larvae			Water quality characteristics on day 0		
	Inserted	Alive at 24 h	% mortality	Diss. oxygen = 7.9 mg/l]	pH = 8.0	Temp=20 ± 2 °C
Control	60	58	3.3			
8 µg a.s./l	30	30	0			
16 µg a.s./L	30	29	3.3			
32 µg a.s./L	30	28	6.7			
64 µg a.s./L	30	9*	70			
128 µg a.s./L	30	0	100			
* larvae laid inactive on bottom of beakers						

Table A7.4.1.2/02-5: Acute toxicity of imidacloprid to chironomids after 24 hours

Test substance	Imidacloprid (tech.) (nominal initial concentrations)
Test object	Larvae of *Chironomus riparius*
Exposure	24 h, static
LC_{50}	55.2 µg as/L
(95 % confidence limits)	48 – 63

Section A7.4.1.2/03 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to Hyalella azteca*

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: Table B.9.2-8, II A, 8.6/18*	
3.	Authors (year)	England, D.; Bucksath, J. D. (1991)	
	Title	Acute toxicity of NTN 33893 to *Hyalella azteca*	
	Company, report No.	Bayer CropScience AG, Report-No.: 101960 BES Ref. : M-007182-01-1	
	Date	1991-10-09	
4.	Testing facility	[redacted]	
5.	Dates of work	June 10, 1991 to June 14, 1991	
6.	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid techn, (Batch-No.: 88R11-19, PF-8543)	
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 FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	
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	Imidacloprid techn, (Batch-No.: 88R11-19, PF-8543), described as a beige to light yellow powder	
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.	
3.1.3	Purity		x
3.1.4	Further relevant properites	none	
3.1.5	Method of analysis	HPLC with UV detection, validated by ABC laboratories in ABC report 37859	
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	
3.3	**Reference substance**	No	

Section A7.4.1.2/03 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to Hyalella azteca*

3.4	**Testing procedure**	x
3.4.1	Dilution water	See Tables A.7.4.1/03-1 to 3. Parameters consistent with EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2 requirements
3.4.2	Test organisms	
3.4.3	Test system	A definitive static acute toxicity study bioassay of imidacloprid was conducted at nominal concentrations of 1. 0.33, 1.0, 3.3, 10, 33, 100, 330, 1000 and 3000 µg as/L. Mean measured concentrations of the test substance were 0.35, 0.97, 3.5, 10, 34, 100, 340, 1000 and 3100 µg as/L, representing 102 ± 2% of nominal. 95.6 to 101.9 % (on average 99.4 %) of nominal.
3.4.4	Test conditions	
3.4.5	Duration of the test	
3.4.6	Test parameter	
3.4.7	Sampling	Mortality and abnormal behaviour were evaluated at 24, 48, 72 and 96 hours.
3.4.8	Monitoring of TS concentration	Yes, 0 and 96h by HPLC
3.4.9	Statistics	LC50 and EC50 by probit analysis or moving average, dose response curve by linear regression
		4 RESULTS
4.1	**Limit Test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal water concentration 0.33, 1.0, 3.3, 10, 33, 100, 330, 1000 and 3000 µg as/L
4.2.2	Actual concentrations of test substance	Mean measured concentrations were 0.35, 0.97, 3.5, 10, 34, 100, 340, 1000 and 3100 µg as/L
4.2.3	Effect data (Immobilisation)	See Table A7.4.1.2/03-4 for effects. Abnormal effects including mortality, lethargy, surfacing and/or immobility were observed at 0.97 µg as/L and higher.
4.2.4	Concentration / response curve	LC50 values could not be calculated at 24 and 48 hours due to insufficient mortality. The 72- and 96-hours LC50 values based on mortality were calculated at 1756 µg as/L (884 – 5448 µg as/L, 95% C.I.) and 526 µg as/L (194 – 1263 µg as/L, 95% C.I.), respectively. EC50 values were calculated for each time period based on combined mortality and immobilization: 218 (148-324 95% C.I.), 129 (85 – 194 95% C.I.), 113 (77-165 95% C.I.) and 55 (34-93 95% C.I.) µg as/L, respectively.
4.2.5	Other effects	
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 A7.4.1.2/03 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to Hyalella azteca*

		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an acute toxicity study conducted according to EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2, *Hyalella azteca* (2-3 mm in size, 2 glass beakers per test concentration with 10 animals each) were exposed to imidacloprid in a static test system at aqueous concentrations of 0, 0.33, 1.0, 3.3, 10, 33, 100, 330, 1000 and 3000 µg as/L (nominal initial). Mean measured concentrations of the test substance were 102 ± 2% of nominal. Mortality and symptoms were reported after 24, 48, 72 and 96 hours.
5.2	**Results and discussion**	Abnormal effects including mortality, lethargy, surfacing and/or immobility were observed at 0.97 µg as/L and higher.
5.2.1	EC_{50}	The 24, 48, 72- and 96-hours EC50 values based on mortality and immobility: 218 (148-324 95% C.I.), 129 (85 – 194 95% C.I.), 113 (77-165 95% C.I.) and 55 (34-93 95% C.I.) µg as/L, respectively.
5.2.2	LC_{50}	The 72- and 96-hours LC50 values based on mortality: 1756 µg as/L (884 – 5448 µg as/L, 95% C.I.) and 526 µg as/L (194 – 1263 µg as/L, 95% C.I.), respectively.
5.2.3	NOEC/LOEC	0.35 µg as/L / 0.97 µg as/L
5.3	**Conclusion**	In 96 hours static toxicity test with *Hyalella azteca,* the 96 hr LC50 was determined to be 526 µg as/L and the 96 hr EC50 was 55 µg as/L . The NOECwas 0.35 µg as/L
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	2006/11/08
Materials and Methods	Applicant's version is acceptable with the following addition/correction: 3.1.3: Purity of test substance not given. 3.4: A definitive static acute toxicity study bioassay of imidacloprid was conducted at nominal concentrations of ~~+~~ 0.33, 1.0, 3.3, 10, 33, 100, 330, 1000 and 3000 µg as/L. Mean measured concentrations of the test substance were 0.35, 0.97, 3.5, 10, 34, 100, 340, 1000 and 3100 µg as/L, representing 102 ± 2% of nominal. The text "95.6 to 101.9 % (on average 99.4 %) of nominal" should be deleted.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-

Section A7.4.1.2/03 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity to Hyalella azteca*

	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 A7.4.1.2/03-1: Test organisms

Criteria	Details
Strain	*Hyallela azteca*
Source	ABC Laboratories in house culture
Age	Test animals 2-3 mm at study initiation
Breeding method	Cultured in hard blended test water
Kind of food	Hard maple leaves supplemented with fish food, cereal leaves and yeast 2 to 3 times weekly
Amount of food	Not detailed, according to guideline
Feeding frequency	Continual leaves during rearing with supplements 2 to 3 times weekly
Pre-treatment	In a controlled temperature bath at approx 20 C with a 16 hour daylight photoperiod
Feeding of animals during test	No

Table A7.4.1.2/03-2: Test system

Criteria	Details
Renewal of test solution	No, static test
Volume of test vessels	Glass beakers prepared with 1 liter test solution
Volume/animal	100 ml
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/03-3: Test conditions

Criteria	Details
Test temperature	20 ± 2 C
Dissolved oxygen	7.3 ± 1.1 mg/l (range 5.2 to 8.2)
pH	8.3 ± 0.1 (range 8.0-8.4)
Adjustment of pH	Not specified
Aeration of dilution water	Not specified
Quality/Intensity of irradiation	Not specified
Photoperiod	16 hour daily
Total hardness	180 mg/l CaCO3
Alkalinity	194 mg/l CaCO3
Conductivity	430 µS/cm

Table A7.4.1.2/03-4: Toxicity of imidacloprid to *Hyalella azteca*

Mean Measured Conc. µg/L	No. Test org.	24 h		48h		72h		96h	
		Cum. mortality	Observa-tions	Cum. mortality	Observa-tions	Cum. mortality	Observa-tions	Cum. mortality	Observa-tions
Control	20	0	20N	0	20N	0	20N	0	20N
0.35	20	0	20N	0	20N	0	20N	0	20N
0.97	20	0	20N	0	20N	0	20N	1	19N
3.5	20	0	20N	0	5Le, 14N, 1Sur	0	4Le, 13N, 3Sur	1	8Le, 9N, 2Sur
10	20	0	3Le, 16N, 1Sur	1	11Le, 8N	1	17Le, 2Sur	1	19Le
34	20	1	1im, 12Le, 2N, 4Sur	1	1im, 14Le, 4Sur	1	1im, 16Le, 2Sur	1	5im, 14Le
100	20	0	4im, 11Le, 5Sur	0	10im, 9Le, 1Sur	0	8im, 12Le	2	6im, 12Le
340	20	1	6im, 8Le, 5Sur	1	12im, 6Le, 1Sur	4	13im, 3Le	8	1im, 1Le
1100	20	0	20im	6	14im	11	9im	16	4im
3100	20	1	19im	3	17im	11	9im	12	8im

N=normal, Im=immobile, Sur=surfacing, Le=lethargic

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity of a potential aquatic degradate of the a.s. to Hyalella azteca*

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: Table B.9.2-8, II A, 8.6/19*	
	Authors (year)	Roney, D. J.; Bowers, L. M. (1996)	
	Title	Acute toxicity of 14C-NTN 33823 to *Hyallela azteca* under static conditions	
	Company, report No.	Bayer CropScience AG, Report-No.: 107315 BES Ref. : M-032758-01-1	
	Date	1996-02-26	
	Testing facility	[redacted]	
	Dates of work	December 4, 1995 to December 8, 1995	
	Test substance(s)	Molecule(s): imidacloprid desnitro	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	x
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	Not specified	
		3 MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	14-C-NTN 33823, 96.9% pure and NTN 33823 hydrochloride salt unlabelled, 2 samples.	
3.1.2	Specification	Sample 1, ref. 940309ELB07, 94.1% purity and 80.2% free base	
3.1.3	Purity	Sample 2, ref. 920716ELB02, 97.7% puirity and 83.3% free base	
3.1.4	Further relevant properites	NTN 33823 estimated water solubility 0.51 g/L at 20 C	
3.1.5	Method of analysis	LSC and HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity of a potential aquatic degradate of the a.s. to Hyalella azteca*

3.3	**Reference substance**	No	
3.4	**Testing procedure**		
3.4.1	Dilution water	See Tables A.7.4.1/04-1 to 3. Parameters consistent with EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2 requirements	
3.4.2	Test organisms		
3.4.3	Test system	A definitive static acute toxicity study bioassay of imidacloprid desnitro (NTN-33823) was conducted at nominal **free base** concentrations of 5.3, 10.7, 21.4, 42.7, and 85.4 mg /L. Mean measured concentrations of the test substance were 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L.	x
3.4.4	Test conditions		
3.4.5	Duration of the test		
3.4.6	Test parameter	Mortality and abnormal behaviour were evaluated at 24, 48, 72 and 96 hours.	
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	Yes, 0 and 96h by HPLC	
3.4.9	Statistics	LC50 and EC50 by probit analysis, LOEC and NOEC determinations by ANOVA followed by Dunnett's test	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal water concentration 0, 5.3, 10.7, 21.4, 42.7, and 85.4 mg /L	
4.2.2	Actual concentrations of test substance	Mean measured concentrations were 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L.	
4.2.3	Effect data (Immobilisation)	See Table A7.4.1.2/04-4 for mortality and effects. Abnormal effect/abnormal position at bottom of test chamber immobility was observed at 11 mg/L and higher.	
4.2.4	Concentration / response curve		
4.2.5	Other effects	Based upon mortality, the 96-hours LC50 value was calculated at 51.8 mg/L (44.0 – 60.9, 95% C.I.). The 96 hour EC50 was 29.0 mg/ L (24.7 – 34.0 mg/L, 95% C.I.). The slope of the toxicity curve as determined by the Probit method was 5.6.	
		The NOEC was 22.1 mg/L and the LOEC was 43.8 mg/L.	
4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	2/10%	
4.3.2	Nature of adverse effects	Mortality at 48h	

Section A7.4.1.2/04 Acute toxicity to invertebrates

Annex Point IIA7.2 *Acute toxicity of a potential aquatic degradate of the a.s. to Hyalella azteca*

4.4	**Test with reference substance**	Not performed
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	In an acute toxicity study conducted according to EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2, *Hyalella azteca* were exposed for 96 hours in a static test system to aqueous concentrations of imidacloprid desnitro at nominal concentrations of 0, 5.3, 10.7, 21.4, 42.7, and 85.4 mg /L. Mean measured concentrations of the test substance were 0, 5.6, 11.0, 22.1, 43.8 and 86.8 mg/L.. Mortality and symptoms were reported after 24, 48, 72 and 96 hours.
5.2	**Results and discussion**	Mortality occurred in controls (2/20), at 43.8 mg/L (6/20) and at 86.8 mg/L (19/20).Abnormal position at bottom of test vessel was noted at 11 mg /L and above.
5.2.1	EC_{50}	96 hour EC50 = 29.0 mg/ L (24.7 – 34.0 mg/L, 95% C.I.).
5.2.2	LC_{50}	96-hours LC50 value = 51.8 mg/L (44.0 – 60.9, 95% C.I.).
5.2.3	NOEC/LOEC	22.1 mg/L / 43.8 mg/L.
5.3	**Conclusion**	In 96 hours static toxicity test with *Hyalella azteca,* the 96 hr LC50 was determined to be 51.8 mg test substance/L and the 96 hr EC50 was 29.0 mg/L. Imidacloprid desnitro, a potential aquatic degradate of the active substance imidacloprid, is 2 orders of magnitude less toxic to *Hyalella azteca* as compared to parent compound.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.2/04	Acute toxicity to invertebrates
Annex Point IIA7.2	*Acute toxicity of a potential aquatic degradate of the a.s. to Hyalella azteca*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2006/11/09
Materials and Methods	Applicant's version is acceptable with the following addition: 2.1: According to the study report, test methods and procedures followed those set forth by USEPA and ASTM 3.4.3: Test system: Each test chamber contained 1.5 g of fine washed silica sand to provide a substrate for the Hyalella.
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.2/04-1: Test organisms

Criteria	Details
Strain	*Hyallela azteca*
Source	BCS in house culture, originally from ABC Laboratories in house culture
Age	Test animals 14 to 21 days at test initiation, head length 0. 2-0.6 mm measured in control at day 4
Breeding method	Cultured in hard blended test water
Kind of food	Hard maple leaves supplemented with fish food
Amount of food	*Ad libitum*
Feeding frequency	*Ad libitum*
Pre-treatment	In a controlled temperature bath at approx 22 C with a 16 hour daylight photoperiod for 3 weeks prior to treatment
Feeding of animals during test	No

Table A7.4.1.2/04-2: Test system

Criteria	Details
Renewal of test solution	No, static test
Volume of test vessels	1L glass crystallization dishes prepared with approx 850 ml test solution
Volume/animal	Approx 85 ml
Number of animals/vessel	10
Number of vessels/ concentration	2
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.2/04-3: Test conditions

Criteria	Details
Test temperature	22 ± 1 C
Dissolved oxygen	Day 0 8.0-8.2 mg/l (92-94% saturation; Day 4 7.6-7.8 mg/L (87-89% saturation)
pH	7.4 to 7.7 over test period
Adjustment of pH	Not specified
Aeration of dilution water	Not during the study
Quality/Intensity of irradiation	705.6 lux
Photoperiod	16 hour daily
Total hardness	Mean of 166 mg/l CaCO3
Alkalinity	120 mg/l CaCO3
Conductivity	425 µmhos

Table A7.4.1.2/04-4: Toxicity of imidacloprid to *Hyalella azteca*

Mean Measured Conc. mg/L	No. Test org.	24 h		48h		72h		96h	
		Cum. mortality	Observa-tions	Cum. mortality	Observa-tions	Cum. mortality	Observa-tions	Cum. mortality	Observa-tions
Control	20	0	20N	2	18N	2	18N	2	18N
5.6	20	0	20N	0	20N	0	20N	0	20N
11.0	20	0	20N	0	20N	0	20N	0	19N 1OB
22.1	20	0	20N	0	20N	0	20N	0	17N 3OB
43.8	20	0	20N	4	10N 6OB	4	7N 9OB	6	2N 12OB
86.8	20	8	5N 7OB	15	1N 4OB	19	1N	19	1OB

N=normal, OB=abnormal position on bottom

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Scenedesmus subspicatus*

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.92.5, II A, 8.2.6 /01*
	Authors (year)	Heimbach, F. (1986a)
	Title	Growth inhibition of green algae (Scenedesmus subspicatus) caused by NTN 33893 (technical)
	Company, report No.	Bayer CropScience AG, Report-No.: HBF/AL 27 BES Ref. : M-006854-01-2
	Date	1986-11-28
	Testing facility	[redacted]
	Dates of work	October 24, 1986 – November 07, 1986
	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**	According to the ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 resp. OECD-Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84)
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	Imidacloprid (NTN 33893) batch no. 2/86, 92.80% purity
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	a.s. in technical grade, IIA IV 4.1, a.s. in water not required by the above mentioned guidelines

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Scenedesmus subspicatus*

3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	
3.3	**Reference substance**	Potassium dichromate	
3.3.1	Method of analysis for reference substance	not required by the above mentioned guidelines	
3.4	**Testing procedure**		x
3.4.1	Culture medium	*Scenedesmus subspicatus* (see Table A7.4.1.3/01-1)	
3.4.2	Test organisms	Study conducted according to ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 resp. OECD-Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84), no deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.3	Test system		
3.4.4	Test conditions		
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	See Tables A7.4.1.3/02-2, 3 and 4	
3.4.7	Sampling		
3.4.8	Monitoring of TS concentration	No, not required by the above mentioned guidelines	
3.4.9	Statistics	regression by Litchfield and Wilcoxon	
		4 RESULTS	
4.1	**Limit Test**	Preliminary study, high doses ranging from 0.1- 10 mg a.s./L Main study 1 dose only	
4.1.1	Concentration	10 mg a.s./l	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	10 mg a.s./L nominal	
4.2.2	Actual concentrations of test substance	Not required by above guideline	
4.2.3	Growth curves	Area under growth curve detailed in Table A7.4.1.3/01-3	
4.2.4	Concentration / response curve	Only 1 concentration was tested in the main study	
4.2.5	Cell concentration data	See Table A7.4.1.3/01-2	

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Scenedesmus subspicatus*

4.2.6	Effect data (cell multiplication inhibition)	No effect at 10 mg a.s./L
4.2.7	Other observed effects	None noted
4.3	**Results of controls**	See Tables A7.4.1.3/01-1, 2 and 3
4.4	**Test with reference substance**	Potassium dichromate
4.4.1	Concentrations	0.18, 0.32, 0.56, 1.00, 1.80 mg a.s./L
4.4.2	Results	1.19 mg/L= E_{r50}, regression line slope s= 2.31 0.44 mg/L=E_bC_{50} , regression line slope s = 2.20

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 resp. OECD-Guideline No. 201 "OECD-Guideline for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84), *Scenedesmus subspicatus* was exposed to imidacloprid under static conditions for 96. 10 mg test substance/L (nominal) was tested.
5.2	**Results and discussion**	No effects were seen in the preliminary study up to and including the highest dose tested, 10 mg a.s./L. As the study author reported difficulties dissolving the product, no higher test concentrations were examined in the definitive test. For the definitive study, no treatment related effects on biomass or growth rate were noted.
5.2.1	NOE_rC	≥10 mg a.s./L
5.2.2	E_{r50}	>10 mg a.s./L
5.2.3	E_bC_{50}	>10 mg a.s./L
5.3	**Conclusion**	In a valid 96 hours limit test with *Scenedesmus subspicatus* the EbC50 and ErC50 of imidacloprid (NTN 33893) were determined to be > 10 mg as/L (nominal).
5.3.1	Reliability	1
5.3.2	Deficiencies	None according to the guidelines of the time of study

Section A7.4.1.3/01 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Scenedesmus subspicatus*

	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 comment: 3.4: 3 replicates for the control and the 10 mg/l test concentration were used.
Results and discussion	Applicant's version is acceptable with the following comment: Table A7.4.1.3/01-2: mean cell number in the contros at 48 h was 22.28 $*10^4$ cells/m.l Table A7.4.1.3/01-3: Area below growth curve for the contol was for 0-72 h: 1682 and for 0-96 h: 6188.
Conclusion	Applicant's version can be adopted.
Reliability	2
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.3/01-1: Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus*
Strain	SAG 86.81
Source	In-house maintained culture
Laboratory culture	Yes
Method of cultivation	Stock cultures grown at 20C under 16 h lt/day in flasks containing no. 1 solution, fresh cultures prepared weekly, precultures of algae inoculated with 10^4 cells/ml and grown in nutrient for 3 days
Pretreatment	No specific details
Initial cell concentration	10^4 cells/ml

Table A7.4.1.3/01-2: Cell concentration data

Test-Substance Concentration (nominal)[1] [mg/l]	Extinctions and cell number (x 10-4)							
	24h		48 h		72 h		96 h	
Control (3 reps, range on ext, avg for cell no)	0.03-0.003	5.87 ± 0.35	0.10-0.11	33.38 ± 1.97	0.40-0.46	88.89 ± 6.85	1.22-1.36	288.60 ± 19.36
pH (day 0, 8.38)	7.92		7.92		9.08		8.15	
10 mg a.s./L (6 reps, range on ext, avg for cell no.)	0.02-0.03	4.99 ± 0.11	0.09-0.10	19.75 ± 1.55	0.37-0.40	78.88 ± 3.55	1.18-1.35	283.82 ± 22.66
pH (day 0, 8.23)	7.95		7.96		9.18		8.07	

Temperature all flasks maintained at 23 ± 1 C (measurements were inadvertently not performed; however, measurements of the temperature in the climatic cabinet indicate 22.8C, within the requirements)

Table A7.4.1.3/01-3: Area (biomass integral) under the curve and % deviation from control (=100%)

Test-Substance Concentration (nominal)[1] [mg/l]	Area (A) under the growth curve and % inhibition							
	0- 24h		0- 48 h		0-72 h		0-96 h	
	A	%	A	%	A	%	A	%
Control	58	100	372	100	1201	100	4442	100
10 mg a.s./L	48	82.0	321	86.2	1480	88.0	5809	93.9

*significantly different

Table A7.4.1.3/01-4: Growth rate and % deviation from control (=100%)

Test-Substance Concentration (nominal)[1] [mg/l]	Growth rate (r) and % inhibition							
	0- 24h		0- 48 h		0-72 h		0-96 h	
	r	%	r	%	r	%	r	%
Control	7.38	100	6.47	100	6.23	100	5.90	100
10 mg a.s./L	6.70	90.8	6.21	96.1	6.07	97.3	5.88	99.7

Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test		X*

*confirmation not required at time of study by the requirement followed; however, the subsequent algal study indicates stability would be expected

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Selenastrum capricornutum*

			Official use only
		1 REFERENCE	
1.1	**Reference**	*PPP monograph: B.9.2.5, II A, 8.2.6 /02*	
	Authors (year)	Dorgerloh, M. (2000)	
	Title	Imidacloprid - Influence on the growth of green alga, Selenastrum capricornutum	
	Company, report No.	Bayer CropScience AG, Report-No.: DOM 20018 BES Ref. : M-033262-01-1	
	Date	2000-05-23	
	Testing facility	[redacted]	
	Dates of work	March 31, 2000 - (biol. part): April 06, 2000; (anal. part): May 05, 2000	
	Test substance(s)	Molecule(s): imidacloprid Substance(s): CONFIDOR	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience AG	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s.for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218	
2.2	**GLP**	Yes (certified laboratory)	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 for imidacloprid technical grade	
3.1.1	Lot/Batch number	Imidacloprid (NTN 33893) as technical grade active substance: purity, 98.6 %., Specification: Article No. 04145852, Pt.-No. 230924394 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	Method 00218, Koenig, validated by Sommer, Appendix C in report	x
3.2	**Preparation of TS solution for poorly soluble or volatile test substance**	Not relevant, not poorly soluble	
3.3	**Reference substance**	Potassium dichromate	

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Selenastrum capricornutum*

3.3.1	Method of analysis for reference substance	Not analytically confirmed
3.4	**Testing procedure**	
3.4.1	Culture medium	*Selenastrum capricornutum*
3.4.2	Test organisms	Study conducted according to EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, no deviations noted by the RMS of the December 2005 91/414 DAR
3.4.3	Test system	
3.4.4	Test conditions	
3.4.5	Duration of the test	72 hours
3.4.6	Test parameter	See Tables A7.4.1.3/02-2, 3 and 4
3.4.7	Sampling	
3.4.8	Monitoring of TS concentration	Yes, day 0 and day 3
3.4.9	Statistics	Probit analysis after Finney, regression by Litchfield and Wilcoxon, analysis of variance by Dunnett's test
	4 RESULTS	
4.1	**Limit Test**	Performed
4.1.1	Concentration	100 mg a.s./l
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	100 mg a.s./L measured concentrations ranged from 100-102% of nominal
4.2.2	Actual concentrations of test substance	
4.2.3	Growth curves	Area under growth curve detailed in Table A7.4.1.3/02-3
4.2.4	Concentration / response curve	Only 1 concentration was tested
4.2.5	Cell concentration data	See Table A7.4.1.3/02-2
4.2.6	Effect data (cell multiplication inhibition)	See Table A7.4.1.3/02-4
4.2.7	Other observed effects	None noted
4.3	**Results of controls**	See Tables A7.4.1.3/02-1, 2 and 3

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Selenastrum capricornutum*

4.4	**Test with reference substance**	Potassium dichromate
4.4.1	Concentrations	0.10, 0.18, 0.32, 0.56, 1.00, 1.80 mg a.s./L
4.4.2	Results	2.34 mg/L= E_{r50} 1.12 mg/L=E_bC_{50}

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to EEC Directive 79/831/E, EG C.3, OECD 201, ISO 8692, ASTM E 1218, *Selenastrum capricornutum* was exposed to imidacloprid under static conditions (shake cultures) for 72h. 100 mg test substance/L (nominal) was tested. Calculations are based on nominal values. The quantities of active substance found at the beginning of the test in reference to the nominal concentrations, were 100 to 102 % (average 101 %). The quantities of active substance found at the end (day 3) were 100 %.
5.2	**Results and discussion**	Growth in the control flasks after 3 days showed a reproduction rate greater than a factor of 16. After 72 hours, the rapid growth of algal cells increased the pH of both control and treated systems. The limit dose of 100 mg a.s./l did have a statistically significant effect on area (biomass integrals) under the growth curve and growth rate.
5.2.1	NOE_rC	<100 mg a.s./L
5.2.2	E_{r50}	>100 mg a.s./L
5.2.3	E_bC_{50}	>100 mg a.s./L
5.3	**Conclusion**	In a valid 72 hours limit test with *Scenedesmus subspicatus* the EbC50 and ErC50 of imidacloprid (NTN 33893) were determined to be > 100 mg as/L (nominal, analytically confirmed).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.3/02 Growth inhibition test on algae

Annex Point IIA7.3 *Growth inhibition of Selenastrum capricornutum*

	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 comment: 3.1.6: Method of analysis: HPLC
Results and discussion	Applicant's version is acceptable.
Conclusion	Applicant's version can be adopted.
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.3/02-1: Test organisms

Criteria	Details
Species	*Selenastrum capricornutum*
Strain	SAG 61.81
Source	Collection of Algal Cultures, Inst. For Plant Physiology, Gottingen, Germany
Laboratory culture	Yes
Method of cultivation	Stock cultures grown at 23 + 2C under 16 h lt/day in cotton plugged ehrlenmeyer flasks containing 50 ml nutrient no. 1 solution, fresh cultures prepared weekly, precultures of algae inoculated with 10^4 cells/ml and grown in 200 ml nutrient no. 2 for 3 days in an incubator
Pretreatment	precultures of algae inoculated with 10^4 cells/ml and grown in 200 ml nutrient no. 2 for 3 days in an incubator before preparation of test solutions
Initial cell concentration	10^4 cells/ml

Table A7.4.1.3/02-2: Cell concentration data

Test-Substance Concentration (nominal)[1] [mg/l]	Extinctions and cell number (x 10-4)					
	24h		48 h		72 h	
Control (6 reps, range on ext, avg for cell no)	0.038-0.041	7.38 ± 0.20	0.155-0.161	30.16 ± 0.79	0.472-0.504	103.9 ± 3.12
pH (day 0, 8.00)	8.16		8.22		8.84	
100 mg a.s./L (6 reps, range on ext, avg for cell no.)	0.038-0.040	7.35 ± 0.15	23.4-28.2	26.68 ± 1.82	60.5-96.0	87.48 ± 13.72
pH (day 0, 7.73)	8.13		8.17		8.77	

Temperature all flasks 23 ± 2 C

Table A7.4.1.3/02-3: Area (biomass integral) under the curve and % deviation from control

Test-Substance Concentration (nominal)[1] [mg/l]	Area (A) under the growth curve and % inhibition					
	0- 24h		0- 48 h		0-72 h	
	A	%	A	%	A	%
Control	77	0.0	506	0.0	2092	0.0
100 mg a.s./L	77	0.5	463	8.4*	1814	13.3*

*significantly different

Table A7.4.1.3/02-4 Growth rate and % deviation from control

Test-Substance Concentration (nominal)[1] [mg/l]	Growth rate (r) and % inhibition					
	0- 24h		0- 48 h		0-72 h	
	r	%	r	%	r	%
Control	2.07	0.0	1.74	0.0	1.57	0.0
100 mg a.s./L	2.07	0.2	1.68	3.6*	1.51	3.8*

*significantly different

Table A7.4.1.3/02-5: Effects on algae average growth rate (based on nominal concentrations)

Test substance	Imidacloprid techn. as
Test object	*Selenastrum capricornutum*
Exposure	72 h, static
EbC_{50} and ErC_{50} (0–72 h) in mg test substance/L	> 100
LOEC in mg test substance/L	= 100
NOEC in mg test substance/L	< 100

Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥80% of initial concentration during test	X	

Section A7.4.1.4/01

Annex Point IIA7.4

Inhibition to microbial activity (aquatic)

Toxicity to bacteria

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.10, II A, 8.7 /01*
	Authors (year)	Mueller; Caspers (2001)
	Title	NTN 33893 - Toxicity to bacteria
	Company, report No.	Bayer CropScience AG, Report-No.: 1058 A/00 B BES Ref. : M-036840-01-1
	Date	2001-02-12
	Testing facility	[redacted]
	Dates of work	January 29, 2001 to February 1, 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**	OECD 209, Commission Directive 88/302/EEC, Official Journal of the EC L 133 Part C
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	NTN 33893, purity 98,4 %, Batch: 230924394
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	Directly weighed into sludge, no confirmatory analysis
3.2	**Preparation of TS solution for poorly soluble or volatile**	Not relevant, not poorly soluble

Section A7.4.1.4/01

Annex Point IIA7.4

Inhibition to microbial activity (aquatic)

Toxicity to bacteria

	test substances		
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not detailed	
3.4	**Testing procedure**		
3.4.1	Culture medium	See Tables A7.4.1.4/01 1 and 2. Conducted according to OECD 209 guideline, no deviations noted by the RMS in the December 2005 91/414 DAR.	x
3.4.2	Inoculum / test organism		
3.4.3	Test system	Activated sludge (waste water treatment plant treating predominantly domestic sewage) was exposed for 3 hours to nominal concentrations ranging from 1000 to 10000 mg test substance/L.	
3.4.4	Test conditions		
3.4.5	Duration of the test		
3.4.6	Test parameter	respiration inhibition	
3.4.7	Analytical parameter	oxygen measurement	
3.4.8	Sampling	0 and 3 hours	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	control without test substance and positive control	
3.4.11	Statistics	EC50 for reference substance by probit; a.s. EC50 is >highest concentration tested	
		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Between 1000 and 10,000 mg/l; See Tables A7.4.1.4/01-3and 4	
4.2.2	Actual concentrations of test substance	Not measured	
4.2.3	Growth curves	Not relevant	
4.2.4	Cell concentration data	Not relevant	
4.2.5	Concentration/ response curve	See Table A7.4.1.4/01-4 for inhibition data	

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Annex Point IIA7.4

Inhibition to microbial activity (aquatic)

Toxicity to bacteria

4.2.6	Effect data	E_{50} >10,000 mg/L
4.2.7	Other observed effects	None noted
4.3	**Results of controls**	See Table A7.4.1.4/01-3 and 4
4.4	**Test with reference substance**	Performed
4.4.1	Concentrations	See Table A7.4.1.4/01-5
4.4.2	Results	E_{50} >10,000 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a study conducted according to OECD 209, Commission Directive 88/302/EEC, Official Journal of the EC L 133 Part C, activated sludge (waste water treatment plant treating predominantly domestic sewage) was exposed for 3 hours to nominal concentrations of imidacloprid ranging from 1000 to 10000 mg test substance/L. 3,5-Dichlorophenol served as the reference substance.
5.2	**Results and discussion**	NTN 33893 showed 27.9 % respiration inhibition of activated sludge at a test substance concentration of 10000 mg/L. The NOEC was 5600 mg as/L (18.4 % inhibition).
5.2.1	EC_{50}	>10,000 mg/L
5.3	**Conclusion**	In a valid study (respiratory rate of controls within 15%, respiratory rate of controls <60 mg O2/l h, EC50 reference compound in the range of 5-30 mg/l) the EC50 for imidacloprid regarding respiration inhibition of activated sludge was determined being greater than 10,000 mg/L. A risk to biological sewage treatment can be excluded.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A7.4.1.4/01

Annex Point IIA7.4

Inhibition to microbial activity (aquatic)

Toxicity to bacteria

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2007/03/02
Materials and Methods	Applicants version is acceptable with the following amendments: 3.4.2 Inoculum/test organismn: Deviation: Because of strong respiration of the activated sludge only 0.36 g/l ss were used. As the preparations with reference substance 3,5-dichlorophenol shows a relationship between concentration of substance and inhibition of the microorganisms and the EC50 (3 h) of 3,5-dichlorophenol is in the accepted range 5 to 30 mg/l, the lower input of inoculum is not considered as affecting the result of the study in a false positive way.
Results and discussion	Applicant's version adopted. NOEC ≥ 5600 mg/l, EC50 > 10000 mg/l.
Conclusion	Applicant's version accepted with the following amendment: A risk to biological sewage treatment can be excluded regarding the results of this study.
Reliability	1
Acceptability	Acceptable.
Remarks	None.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7.4.1.4/01-1: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Species	Not relevant
Strain	Not relevant
Source	sewage treatment plant treating predominantly domestic sewage
Sampling site	aeration tank of waste water plant (Wupper area water authority)
Laboratory culture	No, see above
Method of cultivation	Not relevant
Preparation of inoculum for exposure	Test substance added to 130 ml deionized water and stirred overnight before testing (equilibration stage)
Pretreatment	Aeration of the activated sludge; daily feed with synthetic medium

Table A7.4.1.4/01-2: Test conditions

Criteria	Details
Test temperature	20 ± 2C
pH	6.9
Aeration of dilution water	Not indicated
Suspended solids concentration	360 mg/l suspended solids

Table A7.4.1.4/01-3: Measured values of test substance and control

	Test concentration (mg/l)	O2 start (mg O2/L)	O2 end (mg O2/L)	Time (min)	Temp ©	pH
Test substance	**1000**	**6.2**	**2.9**	**7**	**19.7**	**8.2**
	1800	**6.5**	**2.9**	**8**	**19.7**	**8.2**
	3200	**6.3**	**3.2**	**7**	**19.7**	**8.3**
	5600	**6.5**	**3.6**	**7**	**19.8**	**8.3**
	10,000	**6.3**	**4.1**	**6**	**19.9**	**8.2**
Control 1 (at start)		**5.7**	**2.6**	**6**	**19.6**	**8.1**
Control 2 (at end)		**6.2**	**2.7**	**7**	**19.7**	**8.2**
Physico chemical oxygen control	**10,000**	**8.6**	**8.6**	**9**	**19.5**	**7.4**

Table A7.4.1.4/01-4: Results Test Substance

Test concentration nominal (mg/l)	Respiratory rate (mg/l h)	Pyhs-chem O2-consumption	Respiratory rate – phys-chem O2-consumption (mg/l h	Inhibition (%)
1000	28.3	0.0*	28.3	7.2
1800	27.0	0.0*	27.0	11.5
3200	26.6	0.0*	26.6	12.8
5600	24.9	0.0*	24.9	18.4
10,000	22.0	0.0	22.0	27.9
Control 1	31.0			
Control 2	30.0			

* only determined at 10,000 mg.l concentration

Table A7.4.1.4/01-5: Results Control 3,5-Dichlorophenol

Test concentration (mg/L)	Respiratory rate (mg/l h)	Inhibition (%)
2.5	26.3	13.8
5	27.0	11.5
10	19.7	35.4
20	10.0	67.2
40	6.0	80.3

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

Official use only

1 REFERENCE

1.1	**Reference**	*PPP monograph: B.9.2.2, II A, 8.2.2.2 /01*
	Authors (year)	[redacted] (2002)
	Title	Imidacloprid (NTN 33893): Early life-stage toxicity test with rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions
	Company, report No.	Bayer CropScience AG, Report-No.: 1022.016.321 BES Ref. : M-049894-01-1
	Date	2002-08-29
	Testing facility	[redacted]
	Dates of work	June, 2001 - April 18, 2002
	Test substance(s)	Molecule(s): imidacloprid Substance(s): Imidacloprid (Batch-No.: 2301243968)
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. the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD 210, U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400
2.2	**GLP**	Yes (certified laboratory)
2.3	**Deviations**	No GLP deviations. Deviations from protocol: Based on the requirements of the US EPA-FIFRA § 72-4 the temperature for larval and fish exposure was intended to be 10 ± 1 °C. The continuously measured min/max temperature ranged in practice 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.

3 METHOD

3.1	**Test material**	As given in section 2
3.1.1	Lot/Batch number	Imidacloprid technical (purity: 98.2 %, specification: batch No. 230124368, TOX: 5904-00).
3.1.2	Specification	Specification as given in section 2; stability guaranteed for the duration of the study.
3.1.3	Purity	

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

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 included 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 OECD 210, U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400, no water, handling of embryos, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR	
3.4.2	Test organisms	*Oncorhynchus mykiss* (see Table A7.4. 3.2/01-1)	
3.4.3	Handling of embryos and larvae (OECD 210/212)	Study conducted in accordance with OECD 210, U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400, no water, handling of embryos, test system, conditions, parameters or sampling deviations noted by the RMS of the December 2005 91/414 DAR	x
3.4.4	Test system		
3.4.5	Test conditions		
3.4.6	Duration of the test	91 days	
3.4.7	Test parameter(s)	Water quality parameters (temperature, pH, oxygen) and test substance concentrations were measured weekly	
3.4.8	Examination / Sampling	Biological observations according to guideline; a brief summary: Eggs: day 1 to 37 after start of exposure to determine live, dead and unaccounted for eggs; % fertilized eggs determined on day 12; start of hatching and completion considered 2 distinct entities; percentage hatch calculated as number total fry in each egg cup considering fertilization rate Fry: day 37, number of live, deformed, dead and unaccounted for fry for each egg cup Day 37 to study end, daily observations for: mortality, sublethal effects Day 60 after sacrifice: fish length, wet weight and dry weight Also see Results for more details	
3.4.9	Monitoring of TS concentration	Yes, weekly	
3.4.10	Statistics	Shapiro Wilk's test, Bartlet's test, Dunnett's test, William's test, Kruksal Wallis test	

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

4 RESULTS

4.1	**Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal test concentrations of 0.1, 0.3, 1.0, 3.0, 9.0, and 27.0 mg test substance/L.
4.2.2	Actual concentrations of test substance	Analytical measurements ranged from 89 to 113 percent of the mean measured concentrations during the test at all test levels. The mean measured concentrations were 0.0994, 0.307, 0.977, 3.14, 9.02 and 26.9 mg as/L.
4.2.3	Effect data	See Tables A7.4.3.2/01-2 and 3. NOEC/LOEC values were obtained by observations and statistical analysis as performed with the Williams test (p = 0.05):
4.2.4	Concentration / response curve	See Table A7.4.3.2/01-2 for mortality data. No treatment related increase in mortality was observed.
4.2.5	Other effects	No test item behavioural changes noted, no abnormal morphology
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	See Table A7.4.3.2/01-2 for mortality data.
4.3.2	Nature of effect	Control mortality of 3.3%
4.4	**Test with reference substance**	Not performed

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In a fish early life-stage study conducted according to OECD 210, U.S.-EPA-FIFRA § 72-4, OPPTS 850.1400 guidelines, rainbow trout (*Oncorhynchus mykiss*) were exposed to imidacloprid at mean measured concentrations were 0.0994, 0.307, 0.977, 3.14, 9.02 and 26.9 mg as/L from the day of fertilization of eggs (5 to 5.5 hours after fertilization) through 60 days post hatch (total of 91 days).

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

5.2	**Results and discussion**	Twelve days after start of exposure 4 control egg cups with 50 eggs each were investigated for development of embryos. The fertilization rate was 90%. **Time to hatch and hatching rate:** On day 31, hatching was ≥90% of the viable eggs in control, defining day 0 for the post hatch period. In the 26.9 mg a.s./L group, hatching started earlier; the onset of first hatch in this group was significantly different compared to control and thus the NOEC for the endpoint day of first hatch is 9.02 mg a.s./L. For treated eggs, ≥ 90% of the viable eggs hatched between 29 and 37 days after start of exposure. There were no statistical differences between treated group and control; thus the NOEC for completion of hatch was >26.9 mg a.s./L. No statistically significant differences were found when comparing hatching rates of the treated groups; the NOEC for hatching rate is also >26.9 mg/a.s./L. **Larval deformities and survival:** After completion of hatch no test item related deformities of larvae were observed. No treatment related effect on survival rate was noted. The NOECs for larval deformities and survival are 26.9 mg a.s./L. **Time to swim-up:** Swim-up started day 9 post-hatch. Swim-up in the high dose group started 4 days earlier than the control. In the 26.9 mg a.s./L group, percent swim-up was statistically significantly higher when compared to control on days 40, 42, 43, 44 , 45, 46, 47, 48 and 49. The NOEL for time to swim-up is 9.02 mg a.s/L however, the % swim-up on day 52 (the day control swim was ≥ 95%)was unaffected by treatment; the NOEC for the day of control swim-up is 26.9 mg a.s/L. **Behavioural changes and post hatch survival** No behavioral changes were noted throughout the test, nor was post hatch survival significantly affected at any treatment level. The NOECs for both are 26.9 mg a.s./L. **Growth:** No statistically significant differences in growth parameters (length, wet weight and dry weight) were found between control and treatment groups. The NOEC for all three parameters is 26.9 mg a.s./L. **Deformities**: During the course of the study, one deformity was observed in the 3.14 mg a.s./L replicate A; the fish had a shorter snout region. As no other deformities were noted in higher dose groups, it was concluded that the test item was not the cause and the NOEC for deformities is 26.9 mg a.s./L.
5.2.1	NOEC	The overall NOEC is 9.02 mg a.s./L
5.2.2	LOEC	The overall LOEC is 26.9 mg a.s./L based upon premature hatch and swim-up.
5.3	**Conclusion**	In a 91 days flow-through early life stage test with *Oncorhynchus mykiss* which meets the validity criteria, the overall NOEC of imidacloprid (technical active substance) was determined to be 9.02 mg as/L (mean measured concentration).
5.3.1	Reliability	1
5.3.2	Deficiencies	Deviations: Based on the requirements of the US EPA-FIFRA § 72-4 the temperature for larval and fish exposure was intended to be 10 ± 1 °C. The continuously measured min/max temperature ranged in practice