Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

imidacloprid (ISO); (*E*)-1-(6-chloropyridin-3-ylmethyl)-*N*-nitroimidazolidin-2-ylidenamine

EC Number: 428-040-8
CAS Number: 138261-41-3

CLH-O-0000001412-86-282/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

13 June 2019

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Imidacloprid (ISO);

(E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine

EC Number: -

CAS Number: 138261-41-3

Index Number: 612-252-00-4

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON IMIDACLOPRID (ISO); (*E*)-1-(6-CHLOROPYRIDIN-3-YLMETHYL)-*N*-NITROIMIDAZOLIDIN-2-YLIDENAMINE

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Part A

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Imidacloprid (ISO); (*E*)-1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine
EC number:	-
CAS number:	138261-41-3
Annex VI Index number:	612-252-00-4
Degree of purity:	≥ 97 % (w/w)
Impurities:	Considered confidential, please refer to the confidential annex.

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	Acute Tox. 4*, H302: Harmful if swallowed Aquatic Acute 1, H400 Aquatic Chronic 1, H410
Current proposal for consideration by RAC	Acute Tox. 3, H301: Toxic if swallowed ATE oral: 131 mg/kg bw Aquatic Acute 1, H400 M=100 Aquatic Chronic 1, H410 M=1000
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3, H301: Toxic if swallowed ATE oral: 131 mg/kg bw Aquatic Acute 1, H400 M=100 Aquatic Chronic 1, H410 M=1000

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

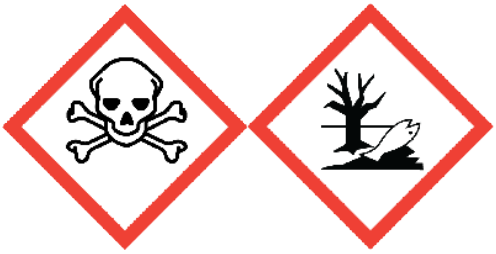
CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None	-	None	Data lacking
2.2.	Flammable gases	None	-	None	Conclusive but not sufficient for classification
2.3.	Flammable aerosols	None	-	None	Conclusive but not sufficient for classification
2.4.	Oxidising gases	None	-	None	Conclusive but not sufficient for classification
2.5.	Gases under pressure	None	-	None	Conclusive but not sufficient for classification
2.6.	Flammable liquids	None	-	None	Conclusive but not sufficient for classification
2.7.	Flammable solids	None	-	None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None	-	None	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	None	-	None	Conclusive but not sufficient for classification
2.10.	Pyrophoric solids	None	-	None	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	None	-	None	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	-	None	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	None	-	None	Conclusive but not sufficient for classification
2.14.	Oxidising solids	None	-	None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None	-	None	Conclusive but not sufficient for classification

2.16.	Substance and mixtures corrosive to metals	None	-	None	Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Acute Tox. 3, H301	-	Acute Tox. 4*, H302	
	Acute toxicity - dermal	None	-	None	Not addressed
	Acute toxicity - inhalation	None	-	None	Not addressed
3.2.	Skin corrosion / irritation	None	-	None	Not addressed
3.3.	Serious eye damage / eye irritation	None	-	None	Not addressed
3.4.	Respiratory sensitisation	None	-	None	Not addressed
3.4.	Skin sensitisation	None	-	None	Not addressed
3.5.	Germ cell mutagenicity	None	-	None	Not addressed
3.6.	Carcinogenicity	None	-	None	Not addressed
3.7.	Reproductive toxicity	None	-	None	Not addressed
3.8.	Specific target organ toxicity –single exposure	None	-	None	Not addressed
3.9.	Specific target organ toxicity – repeated exposure	None	-	None	Not addressed
3.10.	Aspiration hazard	None	-	None	Not addressed
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	M = 100 M = 1000	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	
5.1.	Hazardous to the ozone layer	Not applicable			

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Pictogram: GHS06, GHS09



Signal word: Danger
Hazard statements: H301 Toxic if swallowed
H410 Very toxic to aquatic life with long lasting effects

Proposed notes assigned to an entry: -

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

A harmonised classification for Imidacloprid as Acute Tox. 4*; H302, Aquatic Acute 1; H400 and Aquatic Chronic 1; H410 was introduced to Annex VI of the CLP Regulation with Regulation (EC) Nr. 790/2009 (1. ATP to the CLP Regulation). As the classification was translated from the old harmonized classification under DSD (Directive 2009/2/EC: Xn; N R: 22-50/53) the classification for acute toxicity was included as a minimal classification and no M factors were derived.

2.2 Short summary of the scientific justification for the CLH proposal

The current classification for acute toxicity of Imidacloprid is based on translation of the previous classification under DSD. However, based on available data, classification for acute toxicity category 3 for the oral route is justified. Other toxicological endpoints were not assessed in this report.

Regarding the classification of the environmental hazards, M factors are of high importance in order to ensure the correct classification of plant protection and biocidal products, which is of utmost importance for the authorisation procedure of these products.

Furthermore, new information on aquatic toxicity became available in 2013 that reveals a higher aquatic toxicity than assumed before. Hence, an update of the data basis for the current classification is necessary, mainly influencing the derivation of the M factors.

2.3 Current harmonised classification and labelling

Current legal classification and labelling regarding environmental hazards according to (EC) No 1272/2008 for Imidacloprid is Aquatic Acute 1, H400 “Very toxic to aquatic life” and Aquatic Chronic 1, H410 “Very toxic to aquatic life with long lasting effects”. No harmonised M factors are established.

2.4 Current self-classification and labelling

As of the date of this report the substances has been notified by 105 notifiers to the classification and labelling inventory. Of those 96 classify the substance as “Acute Tox. 4 H302”, “Aquatic Acute 1 H400” and “Aquatic Chronic 1 H410” without giving any M factors or an ATE value. An additional 8 classify the substance, contrary to the legal obligation, as “Acute Tox. 4 H302” and “Aquatic Chronic 1 H410” only. This however may only be to a misinterpretation of the labelling derogations for aquatic hazards. One sole notifier has classified the substances as “Acute Tox. 4 H302”, “Aquatic Acute 1 H400” and “Aquatic Chronic 1 H410” and also derived M factors (Acute: 100; Chronic: 1000) but also stated that the classification was affected by impurities or additives.

RAC general comment
Imidacloprid is an active ingredient in biocidal and plant protection products. Biocidal products containing imidacloprid are intended for professional use (e.g. by pest control operators, farmers), in bait formulations controlling insects such as house flies and cockroaches. The pesticidal product is currently restricted for use as an insecticide to green houses only. Imidacloprid belongs to the family of neonicotinoids and has an existing harmonised classification and labelling in Annex VI to CLP, which was introduced with the first ATP by translation from a previous harmonised classification.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Pursuant to Article 36 paragraph 2 of the CLP regulation active substances in the meaning of Directive 91/414/EEC or Directive 98/8/EC shall be subject to harmonised classification. Imidacloprid is an active substance in the meaning of both directives.

The proposal for the environmental classification considers data that has been submitted by the applicant(s), and were evaluated by the competent authority in the framework of the authorisation of Imidacloprid as biocidal active substance (CAR – Competent Authority Report revised version 2015). As part of this authorisation process, a review of relevant public literature has been carried out.

In addition, studies available in the Draft Assessment Report (DAR 2006) for authorisation as active substances in plant protection products were considered. Hence, all data used for the CLH proposal have previously been evaluated either in the biocidal regulatory process or the pesticides regulatory process.

As of the writing of this report no registrations under Regulation (EC) No 1907/2006 were submitted which could be considered for this report.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

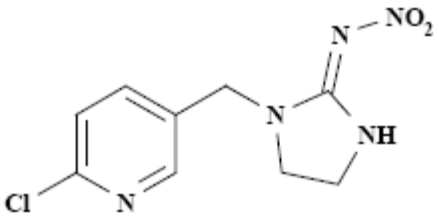
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	-
EC name:	-
CAS number (EC inventory):	138261-41-3
CAS number:	138261-41-3
CAS name:	2-Imidazolidinimine, 1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-, (2E)-
IUPAC name:	(2E)-1-[(6-chloropyridin-3-yl)methyl]-N-nitroimidazolidin-2-imine
CLP Annex VI Index number:	612-252-00-4
Molecular formula:	$C_9H_{10}ClN_5O_2$
Molecular weight range:	255.7 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
(2E)-1-[(6-chloropyridin-3-yl)methyl]-N-nitroimidazolidin-2-imine	98.7 % (w/w)	≥ 97 % (w/w)	

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Considered confidential, please refer to the confidential annex.			

Table 7: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
-				

1.2.1 Composition of test material

Confidential information. Please refer to the confidential annex.

1.3 Physico-chemical properties

Table 8: Summary of physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Solid, crystalline to powder	CA report for a.s. Imidacloprid	visual assessment
Melting/freezing point	144 °C	CA report for a.s. Imidacloprid	experimental result (method: OECD 102 equivalent to 92/69/EEC, A.1 (melt microscope))
Boiling point	not applicable (decomposition above > 200 °C (DTA, 3 K/min) resp. > 230 °C (TGA, 5 K/min)	CA report for a.s. Imidacloprid	experimental result (method: OECD 113)
Relative density	D^{23} = 1.54 D^{20} = 1.41	CA report for a.s. Imidacloprid	experimental result (method: OECD 109 equivalent to 92/69/EEC, A.3 (pycnometer))
Vapour pressure	4 x 10^{-10} Pa at 20 °C 9 x 10^{-10} Pa at 25 °C	CA report for a.s. Imidacloprid	experimental result (method: OECD 102 equivalent to 84/449/EEC, A.4 (vapour pressure balance))
Surface tension	72.20 mN/m at 20 °C (c = 458.91 mg/L)	CA report for a.s. Imidacloprid	experimental result 92/69/EEC, A.5 (ring method)
Water solubility	613 mg/l (unbuffered) 607 mg/l (pH 4) 601 mg/l (pH 9) solubility is independent of pH between 4 and 9	CA report for a.s. Imidacloprid	experimental result (method: OECD 105 equivalent to 84/449/EEC, A.6 (flask method))
Partition coefficient n-octanol/water	log PO/W = 0.57 at 21 °C and pH 7	CA report for a.s. Imidacloprid	experimental result OECD 107 equivalent to 84/449/EEC, A.8 (shaking method)
Flash point	The study does not need to be conducted because the substance is a solid.	expert judgement	study scientifically not necessary
Flammability	Not highly flammable. The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	Mix, K. H. (1993) Report No.: PC635 Bogdoll, B. (2009) (expert statement) Report No.: AF09/040	EEC Method A.10 EEC Methods A.12 and A.13
Explosive properties	non explosive in the sense of the EEC Method A.14 (98,2 % w/w)	Mix, K. H. (1993) Report No.: PC635	EEC Method A.14

Property	Value	Reference	Comment (e.g. measured or estimated)
Explosives according to the criteria given in section 2.1 of Annex I to Regulation (EC) No 1272/2008	Data lacking		A Differential Scanning Calorimetry measurement was used and above 200 °C, a multistage exothermic decomposition was observed; the exothermic decomposition energy was approx. 2100 kJ/kg. Due to this result further tests are required and a testing strategy is proposed as follows: i. Time/pressure test according to UN Test Series 2 in Part I of the Manual of Tests and Criteria ii. BAM Trauzl test (F.3) with initiation by a standard No. 8 detonator (see Appendix 1) according to paragraph 3 "Screening procedures for substances which may have explosive properties" described in Appendix 6 of the UN RTDG, Manual of Tests and Criteria Based on these test results it should be decided if a further detonation test (UN gap test) would be required.
Self-ignition temperature	No spontaneous combustion up to 144 °C (melting point) (98,2 % w/w)	Mix, K. H. (1993) Report No.: PC635	EEC Method A.16
Oxidising properties	no oxidising properties (98,8 % w/w)	Smeykal, H. (2005) Report No.: 20050628.01	EEC Method A.17
Dissociation constant	The substance shows only very weak basic properties. Complete protonation can be achieved only in non-aqueous solvents in the presence of very strong acids. It is not possible to specify a pK value of the substance in pure aqueous system.	CA report for a.s. Imidacloprid	experimental result (method: OECD 112 (Titration method))
Viscosity	-	-	Not determined, substance is a solid.

Data waiving

Information requirement: Flammable gases (including chemically unstable gases)
Reason: Study technically not feasible
Justification: The study does not need to be conducted because the substance is not a gas.

Information requirement: Aerosols
Reason: Study technically not feasible
Justification: The study does not need to be conducted because the substance is no aerosol.

Information requirement: Oxidising gases
Reason: Study technically not feasible
Justification: The study does not need to be conducted because the substance is not a gas.

Information requirement: Gases under pressure
Reason: Study technically not feasible
Justification: The study does not need to be conducted because the substance is not a gas.

Information requirement: Flammable liquids
Reason: Study scientifically unjustified
Justification: The study does not need to be conducted because the substance is a solid.

Information requirement: Self-reactive substances and mixtures
Reason: Study scientifically not necessary
Justification: In spite of the presence of chemical groups in the molecule which are associated with explosive or self-reactive properties, the experimental data by using the differential scanning calorimetry (DSC) shows that the decomposition does not start below or at the melting point, therefore the classification procedure does not need to be applied.

Information requirement: Pyrophoric liquids
Reason: Study technically not feasible
Justification: The study does not need to be conducted because the substance is a solid.

Information requirement: Self-heating substances and mixtures
Reason: Study scientifically not necessary
Justification: The study does not need to be conducted because the substance is completely molten at 160 °C.

Information requirement: Oxidising liquids
Reason: study scientifically not necessary
Justification: The study does not need to be conducted because the substance is a solid.

Information requirement: Organic peroxides
Reason: Study scientifically not necessary
Justification: The study does not need to be conducted because the substance does not fall under the definition of organic peroxides according to GHS and the relevant UN Manual of tests and criteria.

Information requirement: Corrosive to metals
Reason: Study technically not feasible

Justification: The study does not need to be conducted because there is no established suitable test method for solid substances.

2 MANUFACTURE AND USES

Imidacloprid is used as an active substance in biocides and plant protection products. Biocidal products containing Imidacloprid are intended for professional (e.g. by pest control operators, farmers) use in bait formulations controlling insects such as house flies and cockroaches. The pesticide product is currently restricted for use as an insecticide for winter cereals and in green houses only.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
refer to Table 8			

3.2 Physico-chemical properties

3.2.1 Summary and discussion

Experience in handling and use indicates Imidacloprid is not pyrophoric and does not react with water to liberate flammable gases.

Further, it was also tested in a relative self-ignition temperature study (EEC Method A.16) and no spontaneous combustion was found up to 144 °C (melting point).

Imidacloprid has no oxidizing properties in the sense of EEC Method A.17 and no explosive properties in the sense of EEC Method A.14.

3.2.2 Comparison with criteria

3.2.3 Conclusions on classification and labelling

Imidacloprid does not have to be classified as flammable or oxidizing substance or in any of the hazard classes for which a justification for data waiving exist (cf. Table 8).

Due to the data lacking for the hazard classes explosives (section 2.1 of Annex I) and self-reactive substances (section 2.8 of Annex I) the classification cannot be concluded according to the criteria given in these sections to Regulation (EC) No 1272/2008.

4 HUMAN HEALTH HAZARD ASSESSMENT

This CLH dossier is mainly based on the information presented in the assessment reports prepared for the pesticide (DAR 2006) and biocide review procedures but was amended by inclusion of a further unpublished study in rats and of information from the open literature which has not been available or was not taken into consideration before.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not addressed.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In three studies performed under GLP conditions and in accordance with OECD test guideline 401 in rats, Imidacloprid resulted in LD_{50} values between 379 and 648 mg /kg bw.

In an additional study performed by the same laboratory, according to the same guideline, in mice, lower LD_{50} values of 131 and 168 mg/kg bw/d for males and females, respectively, were determined.

Two different batches were tested but the same batch was used in the mouse study as well as in two studies in rats. For this repeatedly used batch, a slightly different purity (94.2 % vs. 94.3 %) has been determined in independent analyses. In all cases, Cremophor EL (2 % v/v in water) was used as vehicle.

The results suggest a higher sensitivity of mice compared to rats.

With regard to the onset of clinical signs and the lowest doses causing mortality in rats, males appeared to be a bit more vulnerable than females.

There is another acute oral study in rats on file with the dossier submitter that was conducted in compliance to the more recent OECD TG 423, i.e., the acute toxic class method. Based on this study, category 4 would be appropriate. Apparently, this study has not been reviewed on EU level so far.

All available acute oral studies are compiled in Table 10.

Table 10: Summary table of available acute oral toxicity studies with Imidacloprid in rodents

Method	Results	Remarks	Reference
Acute oral toxicity in male and female Wistar **rats**, OECD TG 401	LD_{50}: 424 mg/kg bw (males) and 450-475 mg/kg bw (females)	Dose levels tested from 50 (males) or 100 (females) up to 1800 mg/kg bw; clinical signs from 100 mg/kg bw onwards, first dose causing mortality 400 mg/kg bw	Anon 1, 1989a

Method	Results	Remarks	Reference
Acute oral toxicity in male and female Wistar **rats**, OECD TG 401	LD_{50}: 642 mg/kg bw (males) and 648 mg/kg bw (females)	Dose levels tested from 50 (males) or 100 (females) up to 1000 mg/kg bw; clinical signs from 200 mg/kg bw onwards, first dose causing mortality 350 mg/kg bw	Anon 2, 1991a
Acute oral toxicity in male and female Wistar **rats**, OECD TG 401	LD_{50}: 504 mg/kg bw (males) and 379 mg/kg bw (females)	Dose levels tested from 50 (males) or 100 (females) up to 600 or 500 (m/f) mg/kg bw; clinical signs from 200 mg/kg bw onwards, first dose causing mortality 300 mg/kg bw	Anon 3, 1991b
Acute oral toxicity in female Wistar **rats**, OECD TG 423	300 mg/kg bw < LD_{50} < 2000 mg/kg bw	Acute toxic class method, 3 out of 3 rats at 2000 mg/kg bw found dead, no mortality and no signs at 300 mg/kg bw	Anon 4, 2006
Acute oral toxicity in male and female NMRI **mice,** OECD TG 401	LD_{50}: 131 mg/kg bw (males) and 168 mg/kg bw (females)	Dose levels tested from 10 up to 250 mg/kg bw; clinical signs from-71 mg/kg bw and mortality from 100 mg/kg bw onwards	Anon 5, 1989b

In the following, these studies are reported in greater detail. For this purpose, study descriptions were copied from the DAR (Germany, 2006) and slightly amended where necessary. The same studies have been taken into consideration for evaluation of Imidacloprid as a biocide and are reported in the CAR. A description of the study in rats by Mukherjee (2006) has not been found anywhere and, therefore, was amended using the same reporting format as for the previous studies in the DAR.

Studies in rats

Report: Anon 1 (1989a): NTN 33893 - Study for acute oral toxicity to rats. Bayer AG, unpublished report no. 18594.

GLP: Yes

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

Deviations: None

Acceptability: The study is considered acceptable.

Material and Methods:

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

Findings:

Table 11: Dose levels and results in the acute toxicity study of Anon 1 (1989a) in rats

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
Males				
50	0/0/5	-	-	0

Dose [mg/kg bw]	Toxicological results*	Duration of signs	Time of death	Mortality [%]
100	0/5/5	40 m - 1 d	-	0
250	0/5/5	40 m - 1 d	-	0
315	0/5/5	20 m - 1 d	-	0
400	1/5/5	15 m - 2 d	3 h	20
450	4/5/5	25 m - 6 d	2 h - 1 d	80
500	5/5/5	20 m - 7 h	2 h - 7 h	100
1800	5/5/5	15 m - 3 h	1 h - 3 h	100
Females				
100	0/0/5	-	-	0
250	0/5/5	40 m - 1 d	-	0
315	0/5/5	15 m - 2 d	-	0
400	1/5/5	20 m - 2 d	6 h	20
450	0/5/5	25 m - 2 d	-	0
475	5/5/5	30 m - 7 h	2 h - 7 h	100
500	5/5/5	40 m - 6 h	2 h - 6 h	100
1800	5/5/5	15 m - 1 d	2 h - 1 d	100

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

Clinical signs: Apathy and labored breathing were seen at a dose of 100 mg/kg bw. At higher doses, accelerated breathing, decreased motility, staggering gait, narrowed eyelids, trembling and spasms were also observed.

Body weights: Body weight development may have been disturbed by treatment since slight decrements in weight gain were noted on the first 4 days post dosing in animals treated with 250-400 mg/kg bw and above.

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

Conclusion:

Imidacloprid is moderately toxic to rats following acute oral administration. For males, an LD_{50} of 424 mg/kg bw was calculated whereas the LD_{50} in females was between 450 and 475 mg/kg bw.

Report: Anon 2. (1991a): NTN 33893 AMP (proposed c.n.: imidacloprid) – Study for acute oral toxicity in rats. Bayer AG, unpublished report no. 20591.

GLP: Yes

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

Deviations: None

Acceptability: The study is considered acceptable.

Material and Methods:

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

Findings:

Table 12: Dose levels and results in the acute oral toxicity study of Anon 2 (1991a) in rats

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

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

Clinical signs: Apathy, staggering or spastic gait, labored breathing, transient tremor and convulsions, transient or continuing spasms and decreased motility were indicative of neurotoxicity and observed from 200 (in males) or 400 (females) mg/kg bw onwards in all animals, however, to a different extent. In addition, salivation, increased water intake, diuresis, piloerection, and absence of feces were noted.

Body weights: As in the previous study, body weight development may have been disturbed since slight decrements in weight gain were observed within the first 4 days post dosing in animals receiving 200-400 mg/kg bw or more.

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

Conclusion:

Imidacloprid is moderately toxic to rats following acute oral administration. For males, an LD_{50} of 642 mg/kg bw and for females, a nearly identical one of 648 mg/kg bw were calculated.

Report: **Anon 3 (1991b):** NTN 33893 CNS (c.n.: *imidacloprid* (proposed)) - Study for acute oral toxicity to rats. Bayer AG, unpublished report no.: 20637**.**

GLP: Yes

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

Deviations: None

Acceptability: The study is considered acceptable.

Material and Methods:

Imidacloprid (mixed batch 180587, purity: 94.3 %) was formulated in Cremophor® EL / demineralised water (2 % v/v) and administered in a single dose by oral gavage to fasted SPF-bred Wistar rats (Strain Bor: WISW; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany). Again, the group size was five per sex and dose and the same application volume:of 10 mL/kg bw as in the previous studies was chosen.

Findings:

Clinical signs: Apathy, staggering and spastic gait, labored breathing; at higher doses also reduced motility, spasmodic state, periodic tremors, soft faeces and piloerection.

Body weight gain: Slight decrements in weight gain were noted within the first 4 days following administration of 350 mg/kg body weight and of higher doses.

Table 13: Dose levels and results in the acute oral toxicity study of Anon 3 (1991b) in rats

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

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

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

Conclusion:

Imidacloprid is moderately toxic to rats following acute oral administration. For males, an LD_{50} of 504 mg/kg bw was calculated whereas the LD_{50} in females 379 mg/kg bw, i.e., the lowest one that was determined in any of the three rats studies.

Report: Anon 4. (2006): Acute oral toxicity study of imidacloprid technical in rats. Jai Research Foundation, JRF (Vapi, Gujarat, India), for the sponsor Sharda Worldwide Exposrts PVT. Ltd. (Mumbai, India), unpublished JRF study no.:5792.

GLP: Yes

Guideline: OECD 423

Deviations: None

Acceptability: The study is considered acceptable.

Material and Methods:

Imidacloprid (batch SI-06016, purity: 98.56 %) was dissolved in 0.5 % aqueous carboxymethyl cellulose and administered to 3 female Wistar rats (obtained from the JRF animal breeding facility) by oral gavage at a dose level of 2000 mg/kg bw. The dosing volume was 10 mL/kg bw. Following treatment of the first set of three animals, the administration was repeated on two additional groups of three females each, receiving both the same dose of 300 mg/kg bw.

Findings:

Mortality: In the first group receiving the limit dose of 2000 mg/kg bw, all animals died on the day of dosing. No unscheduled mortality was seen in the two groups receiving the low dose of 300 mg/kg bw.

Clinical signs: Lethargy and tremor were reported to have preceded death at the top dose level. No signs were noted in the rats receiving 300 mg/kg bw.

Body weight gain: At the low dose level, all rates gained weight throughout the 14-day post-observation period.

Gross necropsy: In the rats found dead on day of dosing, mottled livers and congestion of brain and lungs were observed.

Conclusion:

Imidacloprid is moderately toxic to rats following acute oral administration. The LD_{50} was between 300 and 2000 mg/kg bw.

Study in mice

Report: Anon 5(1989b): NTN 33893 - Study for acute oral toxicity to mice. Bayer AG, unpublished report no. 18593.

GLP: Yes

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

Deviations: None

Acceptability: The study is considered acceptable.

Material and Methods:

Imidacloprid (mixed batch 180587, purity: 94.2 %) was formulated in Cremophor® EL / demineralised water (2 % v/v) and administered in a single dose by gavage to groups of five male and five female fasted SPF-bred mice (Strain Bor: NMRI; Breeder Winkelmann Versuchstierzucht GmbH&Co. KG, Germany) per dose. The application volume was 10 mL/kg bw.

Findings:

Clinical signs: Apathy, labored breathing, decreased motility, transient staggering gait, transient trembling and transient spasms.

Body weights: No effects on the body weight development were observed.

Gross necropsy: The following findings were described for animals which died during the observation period: liver pale, occasionally dark; spleen pale, occasionally dark; lung dark, patchy and distended. No test substance-related changes were noted in surving animals at scheduled termination.

Table 14: Dose levels and results in the acute oral toxicity study of Bomann (1989b) in mice

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

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

Conclusion:

Following acute oral administration Imidacloprid was more toxic to mice than to rats. An LD_{50} of 131 mg/kg bw was calculated for male mice whereas the LD_{50} in females was 168 mg/kg bw.

4.2.1.2 Acute toxicity: inhalation

Not addressed.

4.2.1.3 Acute toxicity: dermal

Not addressed.

4.2.1.4 Acute toxicity: other routes

Not addressed.

4.2.2 Human information

As for most pesticides, no information is available on effects in humans due to exposure to the active ingredient, Imidacloprid, itself. According to the DAR (2006), occupational medical surveillance of employees in manufacturing did not reveal indications of adverse effects but there are no more recent data.

However, there are a number of published clinical and forensic case reports on poisoning incidents with various plant protection products containing Imidacloprid. Apart from the report by Proença et al. (2005) that was available for the evaluation of Imidacloprid as a biocide already, these publications have not been taken into consideration by EFSA or ECHA so far.

The actual intake of Imidacloprid in the reported cases is not precisely known but can be estimated, at least, in some instances. Cardiac toxicity seems to be of particular importance and critical for the outcome, rather than neurotoxicity. On balance, these reports suggest a markedly higher toxicity of formulations as compared to the active substance. It is not likely that the symptoms or the fatal outcomes may be attributed to Imidacloprid alone because, if so, acute toxicity in humans would be so much higher than in laboratory rodents that this is hardly conceivable. Toxicity and in particular irritation/corrosivity of solvents must be also considered. In particular, a strong contribution of the solvent *N*-methyl pyrrolidone is likely to which exposure was confirmed in at least two of the reported clinical cases whereas it is not known whether it had been ingested or inhaled in the other poisoning incidents.

A lethal case of poisoning was reported from Taiwan by Huang et al. (2006). A woman of 69 years ingested ca 19.2 mg Imidacloprid in 200 mL of the insecticide Confidor. The patient had been suffering from hypertension for six years and experienced a stroke 8 months before the suicidal attempt. High systolic blood pressure and pulse rate, sinus tachycardia and a low body temperature where recorded when she arrived in hospital in a disoriented state. She also exhibited vomiting and hidrosis. In the oropharynx, multiple ulcers were found. Following fluid therapy and gastric lavage with activated charcoal, she gradually improved but one hour later developed apnoea and cyanosis, along with a sharp decline in blood pressure, ventricular fibrillation, tachycardia, and cardiac arrest, and eventually died 12 hours later despite intensive medical care because of intractable hypotension and arrhythmia. Assuming a body weight of 50 kg for a Chinese woman (in fact, not reported in the paper), the fatal dose of Imidacloprid would have been in the magnitude of 0.4 mg/kg bw, i.e., by a factor of ca 1000 below the LD_{50} in the rat and 300 times lower than the LD50 in the mouse. Based on this consideration, it is unlikely that this death was actually due to Imidacloprid. The authors themselves assigned cardiac toxicity to Imidacloprid intake but discussed also a pre-disposing contribution of presumed pre-existing cardiovascular disease and the possible role of the solvent, *N*-methyl pyrrolidone. Both aspects appear more relevant with regard to the fatal outcome.

Another fatal case occurred in Iran but this time without known cardiovascular pre-disposition. A previously healthy 35-year old man of 85 kg bw ingested 350 mL of Imidacloprid (more likely: of a formulation containing Imidacloprid) in an attempt to commit suicide (Shadnia and Moghaddam, 2007). At arrival in hospital one hour later, the patient was disorientated. Drowsiness, dizziness, and palpitations had developed, followed later by mydriasis and apnoea. Leucocytosis was apparent. Sodium was high and potassium was low. Again, cardiac toxicity was crucial. After a first cardiopulmonary arrest on the first day, the patient improved but fever, purulent secretion and a ”paracardiac” (pericardial?) infiltrate developed which were assumed by the physicians to have resulted from concomitant infection. Finally, he exhibited bradycardia and died on the 6th day of hospital admission because of another cardiopulmonary arrest. The relative contributions of Imidacloprid or the (unknown) solvents to this clinical course cannot be determined.

Proença et al. (2005) reported two fatal cases from Portugal but with the focus laid on Imidacloprid analysis in post mortem samples. The first case was a man of 33 years who apparently committed suicide and was found dead at home. Pathological examination did not reveal the cause of death or specific findings but was hampered by postmortal changes including autolysis. Imidacloprid was detected in stomach contents (70 mg in 200 mL) and was also found in liver, kidneys and lungs. Blood concentration was 12.5 µg/mL but there was also some ethanol detected. Based on these

findings, the death was attributed to Imidacloprid ingestion but it was not reported whether analysis for other toxic substances was performed. In the second case of a 66-year old man who was also found dead at home, suicide by ingestion of an insecticide containing Imidacloprid was further substantiated by finding of an empty bottle of Confidor® on the scene. Imidacloprid concentrations in stomach content (37.1 mg in 150 mL), in blood (2 µg/mL) and in the three organs were lower but, this time, Imidacloprid was detected also in urine (0.29 µg/mL) and there were pathological findings. There were corrosive alterations in the upper gastro-intestinal tract, pulmonary edema and the liver was yellow. Again, no information of parallel or previous intake of other substances is available and a possible impact of co-formulants in the ingested plant protection products has not been considered.

In the following cases, the patients survived.

An amount of ca 100 mL of a plant protection product containing 9.6 % imidacloprid, along with < 2 % of an unknown surfactant and > 88 % of the solvent *N*-methyl pyrrolidone, was ingested in a suicide attempt by a 64 years old man in Taiwan. Symptoms included disorientation, drowsiness, dizziness, and palpitations but also cough, vomiting, fever, and abdominal pain. Ulceration of the upper gastrointestinal tract and haemorrhagic gastritis due to corrosive injury was observed. Clinical pathology revealed leucocytosis and hyperglycemia. Thanks to immediate medical treatment and hospital care, the patient survived and completely recovered (Wu et al., 2001). The authors assigned the corrosive findings to the solvent and doubted that the neurotoxic effects could be due to Imidacloprid because of the low amount ingested and since the findings were different from those in laboratory animals.

Deepu et al. (2007) reported drowsiness, fever, vomiting, tachycardia (followed by bradycardia the other day), and hypokalemia in a 22-year old man from India who had intentionally ingested 30 mL of a formulation containing 17.8 % Imidacloprid. The patient survived and was discharged from hospital after 5 days.

In a non-published company report included in the DAR, a further case is briefly reported: A four year old child tolerated the ingestion of four Lizetan combi rodlets (50 mg imidacloprid per rodlet) with no signs of poisoning or adverse health effects. The amount eaten corresponded to ca 10 mg Imidacloprid per kg body weight (Steffens, 2000).

The only case report following inhalative exposure was published by Agarwal and Srinivas (2007) in a “Letter to the editor” referring to the article of Huang et al. (2006, see above). This time, neurological symptoms were most prominent but there was also evidence of cardiac effects and rhabdomyolysis. A 24 years old Indian farmer had been found in an unconscious state while spraying an insecticide (trade name perhaps “Crop King” of an Indian manufacturer) containing 17.8 % Imidacloprid. Clinical examination in a hospital revealed disorientation and extreme agitation (with high doses of a tranquilizer being without effect), fever, hidrosis, cyanosis, tachycardia, and hypertension. On the other hand, haematology and clinical chemistry did not reveal remarkable alterations. Because of his clinical symptoms, the patient was intubated and mechanically ventilated. On the third day, dark urine and high levels of creatine kinase (10 times above the normal range) were indicative of rhabdomyolysis. Treatment was successfully performed with propofol, hydration and alkaline diuresis. Delirium and weakness persisted until day 6 when the patient could be extubated. Later on, he apparently improved and eventually experienced complete restitution. It should be acknowledged that heart damage may also result in in higher creatine kinase levels in serum.

4.2.3 Summary and discussion of acute toxicity

In a number of acute oral studies, Imidacloprid was moderately toxic to rats whereas mice proved more sensitive. Human experience points to a higher toxicity of formulations. It must be emphasised that only the oral route is considered here in detail with regard to classification.

4.2.4 Comparison with criteria

Classifications limits for categorisation based on acute oral toxicity are as follows:

Category 1: oral $LD_{50} \leq 5$ mg/kg bw;
Category 2: oral $LD_{50} > 5$ but ≤ 50 mg/kg bw;
Category 3: oral $LD_{50} > 50$ but ≤ 300 mg/kg bw;
Category 4: oral $LD_{50} > 300$ but ≤ 2000 mg/kg bw.

The reported oral LD_{50} values obtained in male and female mice of 131 and 168 mg/kg bw fall within the classification limits for Acute Tox. Cat. 3. In principle, the rat is the preferred species for testing of acute toxicity. All reported LD_{50} values obtained in rats fall within the classification limits for Acute Tox. Cat. 4. As the LD_{50} values in male and female mice are very similar, were obtained in the same laboratory performing the rat studies, using the same vehicle as in all three rat studies and the same batch as in two of three rat studies, it can be concluded that the observed differences were due to species differences. In accordance with chapter 3.1.2.3.2 of the "Guidance on the Application of the CLP Criteria" (Version 5.0, July 2017), "classification is based on the lowest ATE value (…) in the most sensitive appropriate species tested.". There is no specific information on species relevance in the context of acute oral toxicity of Imidacloprid that would allow disregarding the findings in mice. There is additional evidence coming from poisoning incidents in humans that acute oral toxicity of Imidacloprid might be of concern.

4.2.5 Conclusions on classification and labelling

Based on the submitted data on acute oral toxicity and according to the criteria of the CLP regulation, it is proposed to classify Imidacloprid as Acute Toxicity Category 3 (Acute Tox. 3, H301). The ATE is 131 mg/kg bw, based on the study in mice.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Animal data

The data on acute oral toxicity presented in the CLH dossier consisted of five oral acute toxicity studies, all according to OECD TG and GLP.

There are three rat studies and one mouse study according to OECD TG 401, which were all conducted at the same laboratory and all four used Cremophor EL (2% v/v water) as a vehicle. An additional rat study according to OECD TG 423 was conducted at a different laboratory, using 0,5% aqueous carboxymethyl cellulose as vehicle.

Three different batches of imidacloprid were tested but the same batch was used in the mouse study and in two rat studies. For this repeatedly used batch a slightly different purity (94.2%

vs 94.3%) has been determined in independent analyses.

Table: *Animal data.*

Guideline **Route, Species** **GLP**	**Species, Strain** **Sex** **No of animals**	**Dose levels** **Frequency of application**	**Results**	**Remarks**	**References**
OECD TG 401 GLP; oral/ gavage; rat; mixed batch 180587, purity 94,2%	Wistar rat, 5/sex in each dosage group, Vehicle: Cremophor EL (2% v/v water)	Dose levels: 50 in males & 100, 250, 315, 400, 450, 500, 1800 mg/kg bw in males & females; in females additionally 475 mg/kg bw were applied; Single application	LD_{50}: 424 mg/kg bw (males) & 450-475 mg/kg bw (females)	clinical signs from 100 mg/kg bw in males & 250 mg/kg bw in females onwards; first dose causing mortality: 400 mg/kg bw in males & females	Anon 1, 1989a
OECD TG 401 GLP; oral/ gavage; rat; batch 17133/90, purity 96%	Wistar rat, 5/sex in each dosage group, Vehicle: Cremophor EL (2% v/v water)	Dose levels: 50, 200, 350, 400, 500, 600, 750, 1000 mg/kg bw in males & 100, 400, 450, 500, 600, 1000 mg/kg bw in females; Single application	LD_{50}: 642 mg/kg bw (males) and 648 mg/kg bw (females)	clinical signs from 200 mg/kg bw in males & 400 mg/kg bw in females onwards; first dose causing mortality: 350 mg/kg bw in males & 450 mg/kg bw in females	Anon 2, 1991a

OECD TG 401 GLP; oral/ gavage; rat; mixed batch 180587, purity 94,3%	Wistar rat, 5/sex in each dosage group, Vehicle: Cremophor EL (2% v/v water)	Dose levels: 50, 200, 300, 350, 400, 500, 600 mg/kg bw in males & 100, 200, 300, 350, 400, 500 mg/kg bw in females; Single application	LD_{50}: 504 mg/kg bw (males) and 379 mg/kg bw (females)	clinical signs from 200 mg/kg bw onwards in males & females; first dose causing mortality: 300 mg/kg bw in males & females	Anon 3, 1991b
OECD TG 423 GLP; oral/ gavage; rat; batch SI-06016, purity 98,56%	Wistar rat, 3 females/ dosage group, One group at 2000 mg/kg bw, two groups at 300 mg/kg bw Vehicle: 0.5% aqueous carboxymethyl cellulose	Dose levels: 300 & 2000 mg/kg bw; Single application	LD_{50} between 300 & 2000 mg/kg bw	At the limit dose of 2000 mg/kg bw all animals died on the day of dosing. No unscheduled mortality in rats at 300 mg/kg bw. Lethargy and tremor preceded mortality at the high dose, no signs were seen at the low dose.	Anon 4, 2006
OECD TG 401 GLP; oral/ gavage; mouse; mixed batch 180587, purity 94,2%	Bor:NMRI mice, 5/sex in each dosage group, Vehicle: Cremophor EL (2% v/v water)	Dose levels: 10, 71, 100, 120, 140, 160, 250 mg/kg bw in males & 10, 100, 120, 140, 160, 250 mg/kg bw in females; Single application	LD_{50}: 131 mg/kg bw (males) and 168 mg/kg bw (females)	clinical signs from 71 mg/kg bw in males & 100 mg/kg bw in females; first dose causing mortality: 100 mg/kg bw in males & 120 mg/kg bw in females.	Anon 5, 1989b

While all rat studies support a classification as Acute Tox. 4, the single mouse study supports a classification as Acute Tox. 3. The dossier submitter (DS) concluded that the mouse was more

sensitive than the rat and that male rats were more sensitive than female rats, based on the observed clinical findings and LD_{50} values in males and females.

Clinical signs consisted among others of apathy, laboured breathing, accelerated breathing, decreased motility, staggering gait, narrowed eyelids, trembling and spasms, transient tremor and convulsions, transient or continuing spasms, salivation, increased water intake, diuresis, piloerection and absence of faeces. Some of these effects are indicative of neurotoxicity.

At necropsy, no test substance-related changes were noted in surviving animals which were killed at the end of the observation period.

No studies for dermal and inhalation route are available.

Human data

As for most pesticides, no information is available on effects in humans due to exposure to the active ingredient, imidacloprid, itself. The DS stated that according to the DAR (2006), occupational medical surveillance of employees in manufacturing the substance did not reveal indications of adverse effects but there are no more recent data.

However, the CLH report lists a number of published clinical and forensic case reports on poisoning incidents with various plant protection products containing imidacloprid. The DS also mentions a report by Proença *et al.* (2005), which has been available for the evaluation of imidacloprid as a biocide already, but had not been taken into consideration by EFSA or ECHA so far.

The data consist of eight poisoning cases, four of which were lethal and four in which the patients survived (see tables in the Background Document). The actual intake of imidacloprid in these cases is not precisely known, but it can be roughly estimated, at least for some cases.

Table: *Lethal poisoning cases*

Case	Exposure	Results & remarks	References
69 year old woman from Taiwan, suicide attempt. She had been suffering from hypertension for six years and experienced a stroke 8 months prior to the suicide attempt.	19.2 mg imidacloprid in 200 ml of insecticide Confidor, oral	At delivery to hospital: high systolic blood pressure & pulse rate, sinus tachycardia and a low body temperature, disoriented state, vomiting & hidrosis. Multiple ulcers in the oropharynx. Following fluid therapy and gastric lavage (activated char coal) gradual improvement, but one hour later she developed cyanosis, along with sharp decline in blood pressure, ventricular fibrillation, tachycardia, and cardiac arrest, and eventually died 12 hours later due to intractable hypotension and arrhythmia. The authors assigned cardiac toxicity to imidacloprid intake, but discussed a pre-disposing contribution of presumed pre-existing cardiovascular disease and the possible role of the solvent, N-	Huang *et al.*, 2006

		methyl pyrrolidone. The DS concluded that both aspects appear relevant with regard to the fatal outcome. Though not reported by Huang *et al.* (2006), a fatal dose of imidacloprid of 0.4 mg/kg bw could be calculated, when assuming a body weight of 50 kg for a Chinese woman, as proposed by the DS. This dose would be a factor of ca. 1000 below the rat LD_{50} and 300 times lower than the mouse LD_{50}. The DS concluded that based on this consideration, it was unlikely that this death was actually due to imidacloprid.	
35 year old man from Iran, 85 kg, suicide attempt. No cardiovascular pre-disposition	350 mL imidacloprid (more likely: of a formulation containing imidacloprid), oral ingestion	At delivery to hospital, one hour after ingestion: disoriented, drowsiness, dizziness, palpitations, followed by mydriasis and apnoea. Leucocytosis, increased sodium, decreased potassium. Cardiac toxicity – after a first cardiopulmonary arrest on the first day, the patient improved, but fever, purulent secretion and "paracardiac" (pericardial?) infiltrate developed, which possibly resulted from a concomitant infection. Bradycardia developed and the patient died on the 6^{th} day post admission to hospital. Relative contributions of imidacloprid or the (unknown) solvents cannot be determined.	Shadnia and Moghaddam, 2007

33 year old man from Portugal, found dead, presumably after a suicide attempt.	- oral	Pathological examination did not reveal the cause of death or specific findings but was hampered by postmortal changes including autolysis. Imidacloprid detected in stomach contents (70 mg in 200 mL) and was also found in liver, kidneys and lungs. Blood concentration was 12,5 µL/mL, but there was also some ethanol detected. The death was attributed to imidacloprid ingestion, but it was not reported whether analysis for other toxic substances was also performed.	Proença *et al.*, 2005
66 year old man from Portugal, found dead, presumably after a suicide attempt.	Insecticide containing imidacloprid, confirmed by an empty bottle of Confidor on the scene, oral	Imidacloprid concentrations in stomach content was 37.1 mg in 150 mL, in blood 2 µg/mL and in the three organs were lower but, this time, imidacloprid was detected also in urine at 0,29 µg/mL and there were pathological findings: corrosive alterations in the upper gastro-intestinal tract, pulmonary oedema and the liver was yellow. No information of parallel or previous intake of other substances. No information on possible impact of co-formulants in the ingested plant protection products.	Proença *et al.*, 2005

Table: *Non-lethal poisoning cases*

Case	**Exposure**	**Results & remarks**	**References**
64 year old man from Taiwan, suicide attempt.	ca. 100 mL of a plant protection product containing 9.6% imidacloprid, < 2% of an unknown surfactant and > 88% of the solvent *N*-methyl pyrrolidone (NMP)	Symptoms included disorientation, drowsiness, dizziness, and palpitations but also cough, vomiting, fever, and abdominal pain. Ulceration of the upper gastrointestinal tract and haemorrhagic gastritis due to corrosive injury was observed. Clinical pathology revealed leucocytosis and hyperglycaemia. Thanks to immediate medical treatment and hospital care, the patient survived and completely	Wu *et al.*, 2001

		recovered. The authors assigned the corrosive findings to the solvent and doubted that the neurotoxic effects could be due to imidacloprid because of the low amount ingested and since the findings were different from those in laboratory animals.	
22 year old man from India, intentional ingestion	30 ml of a formulation containing 17,8% imidacloprid	Drowsiness, fever, vomiting, tachycardia (followed by bradycardia the other day) and hypokalaemia. The patient survived and was discharged from hospital after 5 days.	Deepu *et al.* 2007
4 year old child	Ingestion of four Lizetan combi rodlets / 50 mg imidacloprid per rodlet	No signs of poisoning or adverse health effects. The amount taken up corresponded to ca. 10 mg/kg bw imidacloprid	Steffens, 2000, (unpublished company report, included in the DAR)
24 year old farmer, India, found in unconscious state "while" spraying an insecticide – possible trade name: "Crop King" of an Indian manufacturer	Inhalation of insecticide – possible trade name: "Crop King" of an Indian manufacturer, containing 17,8% imidacloprid	Neurological symptoms most prominent. Also evidence of cardiac effects and rhabdomyolysis. At clinical examination in a hospital: disorientation, extreme agitation (with high doses of a tranquiliser being without effect), fever, hidrosis, cyanosis, tachycardia, and hypertension. Haematology and clinical chemistry did not reveal remarkable alterations. Because of his clinical symptoms, the patient was intubated and mechanically ventilated. On the third day, dark urine and high levels of creatine kinase (10 times above the normal range) were indicative of rhabdomyolysis. ➔ It should be acknowledged that heart damage may also result in in higher creatine kinase levels in serum. Treatment was successfully	Agarwal and Srinivas, 2007

		performed with propofol, hydration and alkaline diuresis. Delirium and weakness persisted until day 6 when the patient could be extubated. Later on, he apparently improved and eventually experienced complete restitution.	

Overall, the DS concluded that cardiac toxicity seems to be of particular importance and critical for the outcome of the reported human poisoning cases, neurotoxicity seems less important. The dossier submitter further concluded that the toxicity observed in these cases appears to be considerably stronger than in experimental animals and traces this back to irritant/corrosive solvents (like e.g. N-methyl pyrrolidone, NMP) present in the formulations, which were ingested or inhaled in the human poisoning cases.

The dossier submitter was of the view that the constituents contained in the formulation are responsible for the more severe toxicity seen in humans, rather than assuming a higher sensitivity of humans towards imidacloprid. The difference between humans and experimental animal species would be so big that this would be hardly conceivable.

The DS concluded that imidacloprid was moderately toxic to rats whereas mice proved more sensitive. Human experience points to higher toxicity of formulations. The DS mentioned that the irritant/corrosive properties of solvents such as N-methyl pyrrolidone (NMP) could be responsible for the higher toxicity of formulations. They emphasised that only the oral route was considered in detail for classification.

Based on the lowest LD_{50} of 131 mg/kg bw, as determined in male mice, the DS proposed to classify imidacloprid as Acute Tox. 3; H302, with an ATE of 131 mg/kg bw. They also argued that according to the CLP Regulation, the classification should be based on the lowest determined LD_{50}, if coming from a reliable study. The mouse study was carried out at the same laboratory, using the same vehicle as in all three rat studies and using the same batch as in two of the rat studies, which further supports that mice have a higher sensitivity towards imidacloprid than rat. The LD_{50} of 131 mg/kg bw in males is close to the LD_{50} of 168 mg/kg bw in female mice, further supporting that the mouse is more sensitive than the rat. There is no information available that the mouse would be less relevant for the assessment of imidacloprid's acute toxicity. The dossier submitter concluded that the human poisoning cases were supportive only, as they all resulted from imidacloprid up-take of formulations, which contain solvents, which are likely to have increased the toxicity of the formulation compared to pure imidacloprid.

Comments received during public consultation

During the public consultation one comment was received from the Manufacturer in favour of keeping the classification in Category 4 including the * for minimum classification. In their comment, the Manufacturer described that based on the studies available at that time imidacloprid was classified as R22 (harmful if swallowed) included in the 31st adaptation to technical progress (CD 2009/2/EC). This classification was later translated into a classification according the CLP Regulation as Acute Tox. 4*. The Manufacturer stated that an asterisk was

added to mark it as a minimum classification in view of the available data from the mice study, indicating higher toxicity. They also stated that the DS based their proposal to change from Acute Tox. Category 4* to Category 3 on (a) that there is no information in the guidance on species relevance that would allow disregarding the finding in mice and (b) additional evidence coming from poisoning incidents in humans that acute oral toxicity of imidacloprid might be of concern.

The manufacturer was of the view that the current minimum classification would be in place to cover the existence of data which show higher toxicity (i.e. the mouse data would not be disregarded as recommended by the guidance).

The manufacturer also stated that the DS' proposal to change classification to Acute Tox. 3 was based on human poisoning cases. They listed several drawbacks of the human data including too low intake amounts, exposure to formulations with effects from other constituents, lacking information on intake amounts and other relevant information and that for one case no signs of intoxication were evident.

The DS responded that the current classification proposal is based on the results of a valid oral acute toxicity study in the mouse, which demonstrates that this species is more sensitive than the rat. In line with the manufacturer, the dossier submitter was of the opinion that the current guidance document does not say that a certain species would not be relevant. The dossier submitter also supported the Manufacturers view that the human poisoning incidents should not have an impact on the classification in this case.

RAC agrees with the DS' response.

Assessment and comparison with the classification criteria

RAC concludes that the available animal studies clearly indicate that imidacloprid is acutely toxic via the oral route. While the rat data would support a classification in Category 4, the single mouse study supports a classification in Category 3. As all 5 studies were conducted according to guideline and GLP and had no drawbacks, no difference regarding their suitability for the assessment of classification can be made. As the study in the mouse is no less relevant for humans, the classification has to be based on this, as the most sensitive species., i.e. in line with chapter 3.1.2.3.2 of the "Guidance on the Application of the CLP Criteria" (Version 5.0, July 2017.

RAC also reviewed the human poisoning cases and agrees with the DS and the Manufacturer that they should not have an impact on classification. One major drawback of the available human data is that the exposure was to insecticide formulations, not to pure imidacloprid and that exposure levels could only be roughly estimated. It is further noted that cardiac toxicity was of particular importance in those cases, rather than neurotoxicity and on balance the human cases suggest a markedly higher toxicity of formulations as compared to the active substance. While it might be possible that there are differences regarding the toxicological profile between animals and human, it is rather unlikely that the symptoms or the fatal outcomes may be attributed to imidacloprid alone. If so, acute toxicity in humans would be considerably higher than in animals. Constituents of these mixtures, like solvents with irritant / corrosive or other toxic properties have to be considered and are likely to have contributed to the toxicity of the mixtures.

In conclusion, RAC is of the view that the human poisoning cases indicate that acute toxicity of such formulations is of concern, but they cannot be used to decide on the classification of pure

imidacloprid.

Comparison with the criteria

Based on the lowest LD_{50} value of 131 mg/kg bw derived for male mice in an acute toxicity study in mouse (Anon 5, 1989b), RAC supports a classification as Acute Tox. 3; H301 (LD_{50} > 50 but ≤ 300mg/kg bw) in line with the DS proposal.

RAC also supports to use the same LD_{50} values of 131 mg/kg bw as ATE for imidacloprid.

4.3 Specific target organ toxicity – single exposure

Not addressed.

4.4 Irritation

Not addressed.

4.5 Corrosivity

Not addressed.

4.6 Sensitisation

Not addressed.

4.7 Repeated dose toxicity

Not addressed.

4.8 Specific target organ toxicity – repeated exposure

Not addressed.

4.9 Germ cell mutagenicity (Mutagenicity)

Not addressed.

4.10 Carcinogenicity

Not addressed.

4.11 Toxicity for reproduction

Not addressed.

4.12 Other effects

Not addressed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 15: Summary of relevant information on degradation

Method	Results	Remarks	Reference
US EPA Guideline § 161-1	Half-lives at 12 °C: pH 5 and 7 = stable pH 9 = 2.75 years	Hydrolytic stable	Yoshida, H. (1989)
US EPA § 161-2	Half-lives < 1 day in spring and summer	rapidly photolytic degraded in pure water	Anderson, C. et al. (1991)
Phototransformation of Chemicals in Water, UBA, Germany, Nov. 1990	Using GC-Solar: Half-life: 0.15 - 0.32 days (spring and summer) Half-life: 0.25 - 6.12 days (fall and winter) as function of latitude Using calculation model according to Frank & Kloeppfer: Half-life about 0.2 to 1.6 days in spring/summer Half-life up to 16 days in fall	stepwise photodegradation with oxygen 5 degradation products have been identified	Hellpointner, E. (1990)
OECD Guideline for Testing of Chemicals, Guideline 316: Phototransformation of Chemicals in Water – Direct Photolysis, October 2008	Continuous irradiation: 0.2 d Suntest model: Correlation to natural summer sunlight at 50° N: 0.5 d at 30-40° N: 0.4 d	4 degradation products (not identified) reached levels higher than 10 %	Wehrhahn, A. (2013)
Aerobic aquatic degradation Agriculture Canada 1987	DT_{50} 331days (20 °C), 4.3 % CO_2, metabolites: NTN33893-desnitro + 8 unidentified	Guideline comparable to OECD 309	Stevens, J. et. al. (1997)
Degradation in water-sediment US EPA § 162-4	DT_{50} 32 – 129 days (20 °C), 0.7 % – 2.0 % CO_2, metabolites: NTN33893-5-hydroxy, NTN33893-nitrosimine, NTN33893-urea, chloro nicotinic acid, NTN33893-PEDA, 6-chloro nicotinic acid, NTN33893-desnitro	Three systems tested (orchard drainage ditch, fish pond, pond)	Spiteller, M (1993 Wilmes, R. (1990)
Aerobic soil degradation US EPA 162-1 (1982), BBA IV 4-1 (1986)	DT_{50} 106 – 193 days (20 °C), 4.9 % – 20.3 % CO_2, metabolites: 9 metabolites < 5 %	Five laboratory studies with five German soils	Anderson, C. et.al., (1990a) Anderson, C. and Fritz, R., et.al.(1990a) Anderson, C. and Fritz, R., et.al.(1990b) Hellpointer, E. (1999a)

5.1.1 Stability

Hydrolysis:

Table 16: Hydrolytic degradation

Method / Guideline	pH	Temperature [°C]	Initial TS concentration, C_0 [mol/l]	Reaction rate constant, K_h [1/day]	Half-life, DT_{50m}[h]	Coefficient of correlation, r_2	Reference
US EPA Guideline § 161-1	5	25 °C	5 ppm	stable			Yoshida, H. (1989)
	7	25 °C	5 ppm	stable			
	9	25 °C	5 ppm	1.95×10^{-3}	355	0.8567	

The hydrolysis of Imidacloprid was studied as a function of pH-value. Imidacloprid is stable at pH 5 and 7 and shows slight hydrolysis degradation at pH 9. Not any significant hydrolysis products were determined. The hydrolysis half-life at pH 9, reflecting an average EU outdoor temperature of 285.15 K for fresh water, was calculated by CA to DT_{50} = 2.75 years. In conclusion, hydrolysis is not considered to be a significant degradation route for Imidacloprid at environmentally relevant temperature and pH.

Photolysis in water:

Table 17: Photolysis in water

Method / Guideline	Initial molar TS concentration	Total recovery of test substance [% of appl. a.s.]	Photolysis rate constant (k^c_p)	Direct photolysis sunlight rate constant (k_{pE})	Reaction quantum yield (Φ^c_E)	Half-life ($t_{1/2E}$)	Reference
US EPA § 161-2	5.4 mg/L = 2.11×10^{-2} mmol/L	100.2	experimental: 0.012 min^{-1}	Not calculated	Not relevant	experimental: 57±9 min;	Anderson, C. et al. (1991) A 7.1.1.1.2. /01
			environmental: 0.165 h^{-1}			environmental: 251 min	
"Photo-transformation of Chemicals in Water", UBA, Germany, Nov. 1990	6.31 mg/L = 2.47×10^{-2} mmol/L; 5.18 mg/L = 2.02×10^{-2} mmol/L		average of two experiments: 0.0746 min^{-1}	Calculation model by Frank & Kloeppfer: $0.203 \times 10^{-4} s^{-1}$ (April) $0.289 \times 10^{-4} s^{-1}$ (July) $0.247 \times 10^{-5} s^{-1}$ (November)	average of two experiments: 0.0142	**Frank & Kloeppfer** as function of latitude: 0.2 - 1.6 days (spring and summer) 1.4 - 16 days (fall) **GC solar program:** 0.15 - 0.32 days (spring and summer) 0.25 - 6.12 days (fall and winter) as function of latitude	Hellpointner, E. (1990) A 7.1.1.1.2. /02

Method / Guideline	**Initial molar TS concentration**	**Total recovery of test substance [% of appl. a.s.]**	**Photolysis rate constant (k^c_p)**	**Direct photolysis sunlight rate constant (k_{pE})**	**Reaction quantum yield (Φ^c_E)**	**Half-life ($t_{1/2E}$)**	**Reference**
OECD Guideline for Testing of Chemicals, Guideline 316: Phototransformation of Chemicals in Water – Direct Photolysis, October 2008	0.1 mg/L = $3.91x10^{-4}$ mmol/L	99.9 - 100.2 %	Not specified	3.3862 d^{-1}	0.0123	Continuous irradiation: 0.2 d **Suntest model:** Correlation to natural summer sunlight at 50° N: 0.5 d at 30-40° N: 0.4 d	Wehrhahn, A. (2013) A 7.1.1.1.2. /03

The experimental investigation of photolysis in water shows, that Imidacloprid is rapidly photolytic degraded in pure water with half-lives < 1 day in spring and summer. The photodegradation study by Hellpointner (1990) allowed calculation of environmental half-lives based on reaction quantum yield of 0.0142. Using GC-solar half-lives between 0.15 days (summer 30-50° latitude) and 6.12 days (winter 60° latitude) are estimated in dependence on degree of latitude and seasonal conditions. Applying the model of Frank & Klöpffer environmental half-life yields to about 0.2 to 1.6 days and up to 16 days, in spring/summer and in fall, respectively. Applying the model Suntest estimated half-lives between 0.4 and 0.5 d were obtained in dependence of degree of latitudes 30 – 50 °N (summer). These arithmetic models take only direct photodegradation mechanisms into consideration. However, indirect photodegradation should also contribute to degradation processes in the environment.

During the photodegradation studies the following degradation products were quantitatively identified:

- 17.2 % Imidacloprid guanidine eq. NTN33893-desnitro eq. NTN38014
- 9.8 % 1-[(6-chloro-3-pyridinyl)methyl]-2-imidazolidone eq. NTN33893-urea eq. NTN-33519
- 12.6 % NTN33893-desnitro-olefine
- 15 % 6-chloropicolyl-guanidine eq. NTN 33893-ring-open guanidine and
- 6-chloro-nicotinic acid as degradation product, which could be formed from all identified intermediate degradation products and the parent compound.

The degradation process is suggested as stepwise photodegradation with oxygen.

Phototransformation in air:

Table 18: Phototransformation in air

Guideline / Test method	Time-dependent OH radical concentration [OH radicals cm^{-3}]	Overall reaction rate constant k [cm^3 $molecule^{-1} \times s^{-1}$]	Half-life [h]	Chemical lifetime [h]	Reference
Theoretical estimation according to Atkinson, using US EPA AOPWIN, version 1.87	Global 24-hours-mean concentration of 5×10^5		2.54	3.67	Hellpointner, E. (1999b) A 7.3.1. /01
	12-hours-mean concentration of 1.5×10^6	151.68 x 10^{-12}	0.85	1.2	
No Guideline available, Estimation method by AOPWIN, version 1.91	Global 24-hours-mean concentration of 5×10^5	152.0085 x 10^{-12}	2.53	3.67	C.A. (2006)

Based on half-life as well as chemical lifetime of Imidacloprid, it will be degraded by direct photo-degradation processes in air immediately. Using a 24-hours day and a mean daily OH concentration in air of 5.0×10^5 radicals/cm³, a half-life in air of 2.53 hours for Imidacloprid was calculated.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No estimation of biodegradation was conducted.

5.1.2.2 Screening tests

No screening tests were performed.

5.1.2.3 Simulation tests

5.1.2.3.1 Surface water

Table 19: **Aerobic aquatic degradation**

Method	Test system	Test substance conc.	DT_{50}	Mineralisation	Degradation products	Reference
Agriculture Canada 1987, Guidelines for Determining Environmental Chemistry and Fate of Pesticides, especially Aquatic Aerobic Biotransformation	Pond, Norfolk County, Canada, in dark, 22±1 °C, pH 8.4, 366 days	0.6 mg/L [pyri-dinyl-^{14}C-methylene]-NTN 33893 (imidacloprid)	331 days (20 °C)	4.3% (366 d)	NTN33893-desnitro: max. 26.4 % (274 d)	Stevens, J. et. al. (1997), BES Ref.: M-0244227-02-1III-A7.1.2.2.1

Biodegradation of Imidacloprid was investigated in a study comparable to the relevant OECD guideline 309 (non-sterile, non-light exposed test system) under aerobic aquatic conditions with a non-adapted inoculum. The data show that Imidacloprid disappears slowly in open water systems

with a DT_{50} of 331 days at 20 °C and a mineralisation rate of 4.3 % after 366 d. The test substance was metabolized into nine quantifiable degradation products, among them NTN33893-desnitro, which exceeded 10 % of the initially applied radioactivity. No information is available weather the degradation products are hazardous to the aquatic environment.

5.1.2.3.2 Water-Sediment

Table 20: **Water-sediment degradation studies**

Method	**Test system**	**Test subst. conc.**	**DT_{50}**[1]	**Mine-rali-sation**	**Degradation products**	**Reference**
US EPA § 162-4 (aerobic at 22 ± 1°C in the dark, 30 days)	Stilwell, Kansas,: pond (USA), silty clay: Corg 2.1 %, water: pH 7.63	0.67 mg/L [pyridinyl-^{14}C-methy-lene]-NTN 33893 (imida-cloprid)	129 days (20 °C, total system)	0.7 % (30 d)	NTN33893-5-hydroxy: < 10 % NTN33893-nitrosimine: < 1 % NTN33893-urea: < 1 % chloro nicotinic acid: < 1 %	Spiteller, M. (1993), BES Ref.: M-024398-01-1 A7.1.2.2.2/01
US EPA § 162-4 (aerobic at 22 ± 1°C in the dark, 92 days)	IJzendoorn system (IJS): orchard drainage ditch (NL), loamy silt: Corg 4.09%, water: pH 8.1-8.4 Lienden system (LiS): fish pond (NL), loamy sand: Corg 0.89 %, water: pH 8.1-8.9	0.20 mg/L [pyridinyl-^{14}C-methy-lene]-NTN 33893 (imida-cloprid)	IJS (total system): 30 days (20 °C) LiS (total system): 149.7 days (20 °C)	IJS: 1.4 % (92 d) LiS: 2.0 % (92 d)	IJS: NTN33893-PEDA: < 10 % 6-chloro nicotinic acid: < 10 % NTN33893-desnitro: < 10 % LiS: NTN33893-desnitro: 12.3 % (92 d, total system)	Wilmes, R. (1990), BES Ref.: M-024098-02-1 A7.1.2.2.2/02

[1] Recalculation by eCA according to FOCUS degradation kinetics report (2006) using ModelMaker 4.0

The dissipation behaviour of Imidacloprid applied at a concentration of 20 µg a.s./L for a 10 cm deep water body was studied by Wilmes (1988) in two Dutch water-sediment systems: (a) system IJzendorn, NL and (b) system Lienden, NL). Incubation was carried out under aerobic conditions in the dark at 22 ± 1 °C over a period of 92 days. A third water-sediment system, originating from USA (Stilwell, Kansas) was investigated by Spiteller (1993). The experiment was conducted under aerobic conditions in the dark at 22 ± 1 °C over a period of 30 days. In the study of Wilmes (1988), first order DT_{50}-values (total system) of 32 and 142 days were determined for systems (a) and (b), respectively. A comparable first order DT_{50}-value of 129 days was calculated for the entire system in the study of Spiteller (1993). Since mineralization was < 5 % in all studies, ultimate degradation is considered being slow to negligible.

5.1.2.3.3 Soil

Table 21: **Soil degradation studies**

Method	Test system	Test subst. conc.	DT_{50}[1]	Mineralisation	Degradation products	Reference
Aerobic bare soil						
US EPA 162-1 (1982), BBA IV 4-1 (1986) (aerobic at 20°C in the dark, 100 days)	BBA 2.2: Germany, loamy sand: Corg 2.2 %, water: pH 5.5 moisture: 9.8 %	0.29 mg / kg soil dw [pyridinyl-^{14}C-methylene]-NTN 33893 (imidacloprid)	154 days (20°C)	10.0 %	NTN33893-olefine max 1.8 % (day 100) NTN33893-ring-open-nitroguanidine max 1.8 % (day 100) NTN33893-nitrosimine max 0.8 % (day 35) NTN33893-desnitro max 1.4 % (day 100) NTN33893-urea max 0.3 % (day 62) NTN33893-5-keto-urea, max 1.8 % (day 100) NTN33893-4-keto-urea, max 1.8 % (day 100)	Anderson, C. et.al., (1990a), A7.2.2.1/01
BBA IV 4-1 (1986) (aerobic at 20°C in the dark, 100 days)	Hoefchen: Germany, silt soil: Corg 1.2 % water: pH 5.3 moisture: 9.8 %	0.27 mg / kg soil dw [pyridinyl-^{14}C-methylene]-NTN 33893 (imidacloprid)	193 days (20 °C)	6.4 %		Anderson, C., and Fritz, R., et.al.,(1990a), A7.2.2.1/03
BBA IV 4-1 (1986) (aerobic at 20°C in the dark, 366 days)	Monheim: Germany, sandy loam: Corg 1.3 % water: pH 5.2 moisture: 8.7 %	0.29 mg / kg soil dw [pyridinyl-^{14}C-methylene]-NTN 33893 (imidacloprid)	186 days (20 °C)	4.9 %		Anderson, C., and Fritz, R., et.al.,(1990b), A7.2.2.1/04
BBA IV 4-1 (1986) (aerobic at 20°C in the dark, 126 days)	Laacher Hof-Monheim: Germany, sandy loam: Corg 1.4 % water: pH 6.1 moisture: 17.2 %	0.16 mg / kg soil dw [pyridinyl-^{14}C-methylene]-NTN 33893 (imidacloprid)	106 days (20 °C)	20.3%		Hellpointer, E. (1999a) A7.2.2.1/05
Aerobic special study, with and without groundcover						

Method	**Test system**	**Test subst. conc.**	**DT_{50}[1]**	**Minerali-sation**	**Degradation products**	**Reference**
BBA IV 4-1 (1986) with devia-tions (aerobic at 20°C in the dark, 274 days)	BBA - Hanhofen: Germany, loamy sand with liquid manure and straw: Corg 2.2 % water: pH 5.4	0.23 mg / kg soil dw [pyridinyl-^{14}C-methy-lene]-NTN 33893 (imida-cloprid)	193 days (20 °C, without groundcover)	19.1 %	NTN33893-ring-open-nitroguanidine max 3.4% (day 201) NTN33893-nitrosimine max 1.6% (day 56) NTN33893-desnitro max 4.3% (day 201) NTN3393-6-chloronicotinic acid max 1% (day 56) NTN33893-5-hydroxy max 0.28% (day 201)	Scholz, K. (1992) A7.2.2.4/01

Route and rate of degradation in soil was investigated in five aerobic laboratory studies with European soils at 20 °C in the dark (Table 21). First-order DT_{50} values varied between 106 days and 193 days at 20 °C. Mineralisation (CO_2) was limited, accounting for a maximum of 20.3 % in one sandy loam soil (Laacher Hof, Germany) after 126 days. In total, nine different degradation products have been identified, none exceeding 5 %.

5.1.3 Summary and discussion of degradation

Studies on ready (OECD 301 A-F) and inherent biodegradability (OECD 302 B-C) of Imidacloprid were not performed. For this reason, the degradability of the substance was assessed by considering the results of higher tier biodegradation studies in water, water-sediment, and soil systems as well as abiotic degradation studies (hydrolysis).

In a surface water simulation test the substance was primarily degraded with a half-life of 331 days at 20 °C. Among all simulation studies, the substance was ultimately degraded to a maximum of 20.3 % within 126 days in a soil degradation study.

Imidacloprid is hydrolytically stable under acidic and neutral conditions. In pure water Imidacloprid is rapidly photolytically degraded with half-lives < 1 day in spring and summer. In dependence on degree of latitude and seasonal conditions, half-lives between 0.15 and 6.12 days were estimated. Five degradation products were quantitatively identified. The degradation process is suggested as stepwise photodegradation with oxygen.

Based on the available information, Imidacloprid is considered as not rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 22: Adsorption/desorption

Method /Guideline	Tested Soils	Adsorbed a.s. [%]	K_a[1]	K_{aOC}[2]	K_d[3]	K_{dOC}[4]	K_a / K_d[5]	Degradation products		Reference
								Name	*[%] of a.s.*	
US EPA § 163-1	Sandy loam	21.9	3.69	264	4.12	295	0.90	none		Fritz, R. (1988a)
	Silt soil	21.3	2.44	136	2.76	153	0.88			
	Low humus sandy soil	9.4	1.26	168	2.34	312	0.54			A 7.1.3./01
	Silty clay soil	14.5	1.42	222	2.11	281	0.67			
US EPA § 163-1	Sand	10.9	0.958	411	0.657	282	1.46	none		Williams et.al., (1992)
	Loamy sand	16.6	1.023	293	0.540	154	1.89			
	Silt loam	35.0	4.185	277	4.685	310	0.89			
	Loam	28.9	3.491	301	4.404	379	0.79			A 7.1.3. /02
US EPA § 163-1	Sandy loam Borstel	21.6	1.80	150	3.02	252	0.71	none		Fritz, R. (1993)
	Sandy loam Laacher Hof	29.7	3.17	235	5.16	382	0.61			A 7.1.3. /03
US EPA § 163-1	Sandy loam Borstel	49.7	2.29	176	3.70	284	0.62	none		Fritz, R. (1998b)
	Sandy loam Laacher Hof	41.4	1.70	121	3.34	239	0.51			A 7.1.3. /04
OECD Guideline No. 106	Sandy soil	24.5	1.01	54.8	18.5	1000	0.05	none		Roulstone, P.M (2009)
	Loamy soil	17.7	0.674	63.0	35.7	3340	0.02			
	Silt soil	29.3	1.36	52.0	14.0	534	0.10			A 7.1.3. /05
	Clay loam	37.9	2.28	61.4	13.4	361	0.17			

[1] **K_a = Adsorption coefficient**
[2] K_{aOC} = Adsorption coefficient based on organic carbon content
[3] K_d = Desorption coefficient
[4] K_{dOC} = Desorption coefficient based on organic carbon content
[5] K_a / K_d = Adsorption / Desorption distribution coefficient

Based on the adsorption/desorption studies, Imidacloprid could be classified as being moderately mobile in soil. The arithmetic mean of K_{aOC} is 186.6 mL/g. The value for arithmetic mean of K_{dOC} is 534.9 mL/g. Imidacloprid was stated to be stable during the adsorption/desorption study. Hence, not any relevant transformation products (> 10 % of a.s.) were detected in these studies.

5.2.2 Volatilisation

The vapour pressure of Imidacloprid is 4.0×10^{-10} Pa indicating that the substance is non-volatile. The Henry's constant is 1.677×10^{-10} Pa × m^3 mol^{-1} at 20 °C, therefore Imidacloprid has a low potential of volatilizing from water. The chemical lifetime of Imidacloprid in the troposphere was estimated to be 3.67 hours considering a global 24-hours mean OH-radical concentration. Gathering from these results, accumulation of Imidacloprid in the air is not to be expected.

5.2.3 Distribution modelling

No distribution studies were conducted in addition to the screening methods according to US EPA § 163-1.

5.3 Aquatic Bioaccumulation

Table 23: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Calculation based on the linear model generated by Veith et al. (1979)	BCF = 0.61	Log Kow: 0.57	Guidance on the Biocidal Products Regulation, Volume IV) and Technical Guidance Document Risk Assessment Part III

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

According to the CLP Regulation (EC) No 1272/2008 a log $K_{ow} \geq 4$ is used to indicate a risk for bioaccumulation. The log K_{ow} of Imidacloprid is 0.57. Since the log K_{ow} of Imidacloprid is below the level of concern value of 4, the intrinsic potential for bioaccumulation in aquatic organisms has to be considered to be low.

5.3.1.2 Measured bioaccumulation data

A study on the bioaccumulation behaviour of Imidacloprid is not available.

If measured BCF values are not available, the BCF for fish can be predicted from the relationship between Kow and BCF. For substances with a log Kow of 2-6 the "Guidance on the Biocidal Products Regulation Volume IV" proposes using the linear relationship as developed by Veith et al. (1979).

Imidacloprid has a log Kow < 2 and strictly speaking this equation is not applicable. However, according to the "Technical Guidance Document on Risk Assessment Part III", relevant under the Biocidal Products Directive (BPD 98/8/EG), the same linear model generated by Veith et al., 1979 can be used for substances with a log Kow < 6 for the prediction of BCF. Therefore the calculation of the bioconcentration factor for Imidacloprid in fish on a wet weight basis is as follows:

Log BCFfish = 0.85.log Kow - 0.70

Log BCFfish = 0.85 x 0.57 - 0.70

Log BCFfish = -0.2155

BCFfish = 0.609 $L.Kg_{wetfish}$

5.3.2 Summary and discussion of aquatic bioaccumulation

As measured data on bioaccumulation are not available for Imidacloprid, the assessment of bioaccumulation has to be based on the estimation of bioaccumulation behaviour. Hence, with a log K_{ow} = 0.57 Imidacloprid has to be considered as not bioaccumulative.

5.4 Aquatic toxicity

Table 24: Summary of information most relevant for classification on aquatic toxicity

Method	Results	Remarks	Reference
Fish			
OECD 203 *Oncorhynchus mykiss* Static, 96 h	LC_{50} = 211 mg/L	Results based on nominal concentrations (confirmed by analytical monitoring)	Anonymous (1988b) Report: FF-210 A 7.4.1.1.-01
OECD 210 *Oncorhynchus mykiss* Flow-through, 91 d	NOEC = 9.02 mg/L	Results based on mean measured concentration	Anonymous (2002) Report: 1022.016.321 A 7.4.3.2-01
Invertebrates			
OECD 202 *Chironomus riparius* Static, 24 h	EC_{50} = 0.055 mg/L	Results based on nominal concentrations (confirmed by analytical monitoring)	Dorgerloh, M.; Sommer, H. (2002a) A 7.4.1.2-02
No guideline study ***Cloeon dipterum*** **Static, 96 h**	**EC_{50} = 0.00102 mg/L**	**Results based on nominal concentrations (confirmed by analytical monitoring)**	**Roessink et al. (2013)**
OECD 219 *Chironomus riparius* Static, 28 d	EC_{10} = 0.00087 mg/L	recalculated to mean measured concentrations	Dorgerloh, M.; Sommer, H. (2001a) A 7.4.3.4--02
No guideline study ***Caenis horaria*** **Semi-static, 28 d**	**EC_{10} = 0.000024 mg/L**	**Results based on nominal concentration (confirmed by analytical monitoring)**	**Roessink et al. (2013)**
Algae			
OECD 201 *Selenastrum capricornutum* Static, 72 h	E_rC50 > 10 mg/L NOE_rC < 100 mg/	Limit test with 10/100 mg/L, nominal concentration (confirmed by analytical monitoring)	Dorgerloh, M. (2000) A 7.4.1.3-02

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Table 25: Short-term toxicity to fish

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.i./L]			Remarks	Reference
			design	duration	LC_0	LC_{50}	LC_{100}		
OECD 203	*Oncorhynchus mykiss*	mortality	static	96 h	158	211	281	Results based on nominal concentrations (confirmed by analytical monitoring) RI 1	Anonymous (1988b) Report: FF-210 CAR: A 7.4.1.1. /01
U.S.-EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-1	*Oncorhynchus mykiss*	mortality	static	96 h	> 83	> 83	> 83	Results based on mean measured concentrations RI: 2	Anonymous (1990) Report: 100349 CAR: A 7.4.1.1. /02
EEC DIRECTIVE 79/831/WG, Annex V	*Leuciscus idus*	mortality	static	96 h	> 178	237	316	Results based on nominal concentrations (confirmed by analytical monitoring) RI: 1	Anonymous (1987) Report: FO-1042 CAR: A 7.4.1.1. /04

The acute toxicity of Imidacloprid to fish was tested with two different species (*Oncorhynchus mykiss* and *Leucsicus idus*) in static systems.

In one short-term fish toxicity study (A 7.4.1.1./01) conducted according to OECD 203, young rainbow trout (*Oncorhynchus mykiss*) (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. Analytical monitoring of the test substance concentration revealed stability during the exposure period except for the highest test concentration. Mortality and symptoms of toxicity were reported. Mortality was observed in the two top concentrations (281 and 500 mg as/L). Symptoms of intoxication occurred mainly at 158 mg as/L and higher concentrations. The toxic symptoms were noted as: swimming behaviour slightly irregular (light symptom), apathic, lying on side/ back and staggering. A 96h-LC_{50} of 211 mg/L based on nominal concentrations was determined. Validity criteria were all met.

In another short-term fish toxicity study (A 7.4.1.1. /02) conducted according to EPA Guideline 72-1, young rainbow trout (*Oncorhynchus mykiss*; 10 fish per test concentration) were exposed for 96 hours under static conditions to nominal concentrations of 16, 27, 45, 75 and 125 mg as/L (mean measured concentrations were 15, 27, 42, 64 and 83 mg as/L). No mortalities were recorded up to the highest test concentration. Thus, the 96 hour LC_{50}-values is > 83 mg as/L related to mean

measured concentrations. Dark discoloration, fish on the bottom of the test chamber, erratic swimming and/or quiescence were observed as sublethal effects in the 64 and 83 mg as/L test concentrations during the 96-hour exposure period. O_2 content decreased from 9.1 – 9.3 mg/l at 0 h to 5.8 – 6.8mg/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. However, as the same tendency occurred in both treatment and control groups and no effects (mortality and observations) were observed in the control at any time, the study can be assigned a reliability of 2.

In a further short-term fish toxicity study (A 7.4.1.1. /04) conducted according to EEC directive, 1984, the toxicity of Imidacloprid to Golden Orfe (*Leuciscus idus*) was determined in a 96-h-static test. The nominal concentrations tested were 100, 178, 316, 562 and 1000 mg as/L (nominal). 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. Thus, effect values are based on nominal concentrations. The lowest lethal concentration was 316 mg as/L, and the no-observed-effect-concentration (NOEC) 178 mg as/L. The 96-hour LC_{50} of the technical active substance was determined to be 237 mg as/L with a range from 178 to 316 mg as/L. No sublethal effects were observed in surviving fish. Validity criteria were all met.

5.4.1.2 Long-term toxicity to fish

Table 26: Long-term toxicity to fish

Guideline /Test method	Species	Endpoint /Type of test	Exposure		Results [mg a.i./L]		Remarks	Reference
			design	duration	NOEC	LOEC		
OECD 210	*Oncorhynchus mykiss*	Time to hatch and swim up	Flow-through	91 d	9.02	26.9	Results based on mean measured concentration RI: 1	Anonymous (2002) Report: 1022.016.321 CAR: A 7.4.3.2. /01

In a fish early life-stage study conducted according to OECD 210 (A 7.4.3.2. /01), *Oncorhynchus mykiss* was exposed to Imidacloprid at nominal concentrations of 0.1, 0.3, 1.0, 3.0, 9.0, and 27.0 mg /L, corresponding to mean measured concentrations of 0.0994, 0.307, 0.977, 3.14, 9.02 and 26.9 mg/L from the day of fertilization of eggs (5 to 5.5 hours after fertilization) through 60 days post hatch (total of 91 days). Observed endpoints were time to hatch and hatching rate, larval deformities and survival, time to swim-up, behavioural changes and post-hatch survival and growth.

The most sensitive endpoints were time to hatch and swim up, in the 26.9 mg/L group, hatching and swim up started earlier, the onset of the first hatch and swim up in this group was significantly different compared to the control and thus the NOEC for the endpoint day of first hatch and swim up is 9.02 mg/L. For the other observed endpoints the NOEC was 26.9 mg/L, for the highest concentration tested.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 27: Acute toxicity to invertebrates

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [µg a.i./L]	Remarks	Reference
			design	duration	E/LC_{50}		
No guideline study	*Cloeon dipterum*	immobili-sation	static	96 h	1.02	Results based on nominal conc. Confirmed by analytical monitoring. RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Caenis horaria*	immobili-sation	static	96 h	1.77	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Asellus aquaticus*	immobili-sation	static	96 h	119	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Gammarus pulex*	immobili-sation	static	96 h	18.3	Results based on nominal conc. Confirmed by analytical monitoring, control mortality > 10 %, RI 3 (control mortality 33 %)	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Chaoborus obscuripes*	immobili-sation	static	96 h	284	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Sialis lutaria*	immobili-sation	static	96 h	50.6	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Plea minutissima*	immobili-sation	static	96 h	35.9	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05

Method / Guideline	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [µg a.i./L]**	**Remarks**	**Reference**
			design	**duration**	**E/LC$_{50}$**		
No guideline study	*Notonecta* spp.	immobili-sation	static	96 h	18.2	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Micronecta* spp.	immobili-sation	static	96 h	10.8	Results based on nominal conc. Confirmed by analytical monitoring, control mortality > 10 %, RI: 3 (control mortality 20 %)	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	Limnephilidae	immobili-sation	static	96 h	1.79	Results based on nominal conc. Confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.1.2/05
No guideline study	*Caenis horaria*	immobilisation	static	96 h	6.0	Same experimental setup and species as Roessink et al. (2013), but instead of summer generations, winter generations were tested. RI: 2	Van den Brink et al. (2016)
No guideline study	*Cloeon dipterum*	immobilisation	static	96 h	18	Same experimental setup and species as Roessink et al. (2013), but instead of summer generations, winter generations were tested. RI: 2	Van den Brink et al. (2016)
OECD 202	*Daphnia magna*	Immobili-sation	static	48 h	8.5×10^4	Results based on mean measured conc. RI: 1	Young, B. M.; Hicks, S. L. (1990) CAR: A 7.4.1.2. /01
OECD 202	Chironomus riparius	mortality	static	24 h	55	Results based on nominal conc. (confirmed by analytical monitoring) RI: 1	Dorgerloh, M.; Sommer, H. (2002) CAR: A 7.4.1.2. /02

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [µg a.i./L]	Remarks	Reference
			design	duration	E/LC_{50}		
US EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	*Hyalella azteca*	Mortality immobilty	static	96 h	526 (LC_{50}) 55 (EC_{50})	Results based on mean measured conc. RI: 1	England, D.; Bucksath, J. D. (1991) CAR: A 7.4.1.2. /03
US EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	*Mysidopsis bahia*	mortality	Flow-through	96 h	34	Mean measured conc. RI: 1	DAR: Ward G.S. (1990)*
OECD 202 Test substance: **Major metabolite imidacloprid desnitro**	*Hyalella azteca*	mortality	static	96 h	51.8 (LC_{50}) 29.8 (EC_{50})	Results based on mean measured concentration RI: 1	Roney, D. J.; Bowers, L. M. (1996) CAR: A 7.4.1.2 /04

*Study evaluation based on Draft Assessment Report for Imidacloprid 2006 (can be obtained via http://dar.efsa.europa.eu/dar-web/provision)

For Imidacloprid a range of short-term toxicity tests with a variety of aquatic invertebrates are available. Roessink et al. (2013, A7.4.3.4/04) performed short-term toxicity tests with 10 aquatic invertebrate species from different taxonomic groups. Test organisms were collected from an uncontaminated aquatic ecosystem. Early larval insect instars were used for the tests. The test organisms were acclimated for at least 3 days to laboratory conditions (18 +/- 2 °C, 12:12 hours light: dark). In the acute tests 5 concentrations and a control were tested using 2-3 replicates with each 10 test animals. Exposure period was 96 h. Endpoints were immobilisation and mortality. Imidacloprid concentrations measured in the dosing solution were, on average, 97.5 % of the nominal concentration. In the short-term studies no further analytical monitoring was performed. However, from the analytical monitoring performed for the long-term studies (see 5.4.2.2) it can be concluded that the test substance concentration was stable during the exposure period of 96 h and thus the use of nominal concentrations is justified. The 96 h-EC_{50} values range from 1.02 – 284 µg/L for the endpoint immobilisation. Most sensitive species were *Cloeon dipterum* (1.02 µg/L), *Caenis horaria* (1.77 µg/L) and Limnephilidae (1.79 µg/L). Least sensitive were *Chaoborus obscuripes* (284 µg/L) and *Asellus aquaticus* (119 µg/L). Concerning the validity criteria of OECD TG 202 (*Daphnia* Acute Immobilisation Test) the criterion of 10 % maximum immobilisation in controls is fulfilled for 8 of the 10 tests. For the tests with *Gammarus pulex* and *Micronecta* spp. immobilisation in the controls exceeds this values, leading to a reliability of 3 for these two tests. Also the second validity criterion (dissolved oxygen content > 3 mg/L) was fulfilled for all tests expect the test with *Gammarus pulex.*

In order to investigate the influence of seasonality on the sensitivity of Ephemeroptera species another non-guideline study (van den Brink et al. 2016) with the same experimental setup and species as Roessink et al. (2013) was conducted, but instead of summer generations, winter generations were tested. Short-term toxicity tests were performed for 6 out of the 10 previously

tested species, including the two most sensitive Ephemeroptera species *Caenis horaria* and *Cloeon dipterum*. The acute test 96h-EC_{50} values range from 6.0 – 3258 µg/L for the endpoint immobilization, with *Caenis horaria* being the most and *Chaoborus obscuripes* the least sensitive species. As all short-term toxicity values for the winter generations are higher than for the summer generations, the results indicate that concerning the sensitivity of insect species there are large seasonal differences. For precautionary reasons the lowest toxicity values for the summer generations are considered as most relevant for hazard assessment and will be used for classification.

In addition, short-term toxicity tests with 4 different species of aquatic invertebrates are available that were performed according to standard guidelines. While *Daphnia magna* was by orders less sensitive with a 48h-EC_{50} of 85 mg/L, the results obtained for *Chironomus riparius* (24h-LC_{50} = 55 µg/L) *Hyalella azteca* (96h-EC_{50} = 55 µg/L) and *Mysidopsis bahia* (96h-LC_{50} = 34 µg/L) fit well in the range of the effect values reported by Roessink et al.

Furthermore, the acute toxicity of the metabolite imidacloprid desnitro to *Hyalella azteca* (A 7.4.1.2 /04) was studied in a static test over an exposure period of 96 h in a study according to OECD 202. This aquatic invertebrate was among the most sensitive species to imidacloprid. Mortality and abnormal effect/abnormal position at bottom of test chamber (immobility) were reported after 24, 48, 72 and 96 hours. The 96 h LC_{50} was determined to be 51.8 mg /l and the 96 h EC_{50} was 29.0 mg/L. Imidacloprid desnitro, a potential aquatic degradate of the active substance imidacloprid, is by orders of magnitude less toxic to *Hyalella azteca* as compared to parent compound.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 28: Long-term toxicity to invertebrates

Guideline /Test method	**Species**	**Endpoint / Type of test**	**Exposure**		**Results [µg a.i./L]**		**Remarks**	**Reference**
			design	**duration**	**NOEC**	**LOEC**		
No guideline study	***Cloeon dipterum***	**immobili-sation**	**semi-static**	**28 d**	**0.033**	**0.123**	**Results based on nominal concentration confirmed by analytical monitoring** **RI: 2**	**Roessink et al. (2013)** **CAR: A7.4.3.4/04**
No guideline study	***Caenis horaria***	**immobili-sation**	**semi-static**	**28 d**	**0.024**	**0.126**	**Results based on nominal concentration confirmed by analytical monitoring** **RI: 2**	**Roessink et al. (2013)** **CAR: A7.4.3.4/04**
No guideline study	*Asellus aquaticus*	immobili-sation	semi-static	28 d	1.71	11.9	Results based on nominal concentration confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.3.4/04

Guideline /Test method	Species	Endpoint / Type of test	Exposure		Results [µg a.i./L]		Remarks	Reference
			design	duration	NOEC	LOEC		
No guideline study	*Gammarus pulex*	immobili-sation	semi-static	28 d	2.95	15.4	Results based on nominal concentration confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.3.4/04
No guideline study	*Chaoborus obscuripes*	immobili-sation	semi-static	28 d	4.57	11.8	Results based on nominal concentration confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.3.4/04
No guideline study	*Sialis lutaria*	immobili-sation	semi-static	28 d	1.28	3.46	Results based on nominal concentration confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.3.4/04
No guideline study	*Plea minutissima*	immobili-sation	semi-static	28 d	2.03	6.45	Results based on nominal concentration confirmed by analytical monitoring RI: 2	Roessink et al. (2013) CAR: A7.4.3.4/04
No guideline study	*Cloeon dipterum*	immobilisation	semi-static	28 d	0.4 (EC_{10})	-	Same experimental setup and species as Roessink et al. (2013), but instead of summer generations, winter generations were tested.	Van den Brink et al. (2016)
US EPA-FIFRA 72-4	*Daphnia magna*	Reproduction, survival, length	Semi-static	21 d	1.8×10^3 (length)	3.6 (length)	Results based on mean measured concentrations RI: 1	Young, B. M.; Blakemore, G. C. (1990) CAR: A 7.4.3.4. /01

Long-term toxicity tests were performed by Roessink et al. (2013, A7.4.3.4/04) with aquatic invertebrate species from 7 different taxonomic groups. Test organisms were collected from an uncontaminated aquatic ecosystem. Early larval insect instars were used for the tests. The test organisms were acclimated for at least 3 days to laboratory conditions (18 +/- 2 °C, 12:12 hours light: dark). 5 concentrations and a control were tested using 3 replicates with each 10 test animals. Exposure period was 28 d. Endpoints were immobilisation and mortality. Every week the test solution was renewed and the living animals were transferred to the new test vessels. Imidacloprid concentrations measured in the dosing solution were, on average, 95.5 % of the nominal concentration. Analytical monitoring was performed for the control and the highest test concentration. Samples were collected at the end of each test week. Samples were analysed by liquid chromatography – tandem mass spectrometry. Measured concentrations were in the range of 84.9 – 97 % of the nominal concentration, thus proving the test substance to be stable during the exposure phase. 28d-EC_{10} values (immobilisation) for the 7 tested species were in the range of 0.024 – 4.57 µg/L. As in the short-term studies, the mayflies *Cloeon dipterum* (28d-EC_{10} = 0.033 µg/L) and *Caenis horaria* (28d-EC_{10} = 0.024 µg/L) were most sensitive.

Concerning the non-guideline study by van den Brink et al. (2016, Supplemental data) investigating the influence of seasonality on the sensitivity of Ephemeroptera species, only one long-term test with *Cloeon dipterum* was conducted. The 28d-EC_{10} value (immobilisation) was 0.40 µg/L. As all short-term and long-term toxicity values for the winter generations are higher than for the summer generations, the results indicate that concerning the sensitivity of insect species there are large seasonal differences. For precautionary reasons the lowest toxicity values for the summer generations are considered as most relevant for hazard assessment and will be used for classification.

In addition, a long-term reproduction study with *Daphnia magna* (A 7.4.3.4. /01) is available. The effect of Imidacloprid on the reproduction of *Daphnia magna* was determined in a 21 d study according to US EPA-FIFRA 72-4 guideline under semi-static test conditions. 6 first instars of *Daphnia magna* (< 24 h old) per test chamber (4 replicates) were exposed under static renewal conditions for 21 days to mean measured concentrations of 0.46, 0.86, 1.8, 3.6 and 7.3 mg/L. Percent survival and adult length, young/adult reproduction and time to first brood were measured. Daphnid reproduction and percent survival were significantly affected at 7.3 mg/L. The 21d EC_{50} was estimated to be higher than 7.3 mg/L. Adult daphnid length was significantly affected at 3.6 and 7.3 mg/L. Therefore, a NOEC of 1.8 mg/L was determined for this endpoint.

5.4.3 Algae and aquatic plants

Table 29: Growth inhibition on algae

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.i./L)			Remarks	Reference
			design	duration	NOE$_r$C	E_bC_{50}[1]	E_rC_{50}[2]		
OECD 201	*Scenedesmus subspicatus*	Growth inhibition	Static	96 h	≥ 10	> 10	> 10	Limit test with 10 mg/L; nominal conc. RI: 2	Heimbach, F. (1986a) CAR: A 7.4.1.3. /01
OECD 201	*Selenastrum capricornutum*	Growth inhibition	Static	72 h	< 100	> 100	> 100	Limit test with 100 mg/L,	Dorgerloh, M. (2000)

Method / Guideline	Species	Endpoint / Type of test	Exposure		Results [mg a.i./L)			Remarks	Reference
			design	duration	NOE'C	E_bC_{50}[1]	E_rC_{50}[2]		
								nominal conc. (confirmed by analytical monitoring) RI: 1	CAR: A 7.4.1.3. /02

There are two limit studies with green algae available. Both studies were performed according to OECD 201.

In one study (A 7.4.1.3. /01) conducted according to OECD Guideline No. 201, *Scenedesmus subspicatus* was exposed to Imidacloprid under static conditions for 96 h. 10 mg test substance/L (nominal) was tested. 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. No treatment related effects on biomass or growth rate were observed in the definitive test. Therefore, the 96h-ErC_{50} was > 10 mg/L. As no analytical monitoring was performed, the effect value is based on nominal concentrations. The cell concentration in control cultures increased at least by a factor of 16 within 3 days, thus the validity criterion is fulfilled.

In another study (A 7.4.1.3. /02) 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 for 72 h. 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 %. Cell concentration data show monotone exponential growth during the exposure period and cell concentration in control cultures increased at least by a factor of 16 within 3 days. Thus, the validity criterion is fulfilled. The limit dose of 100 mg/L did have a statistically significant effect on area (biomass integrals) under the growth curve and growth rate, but this effect was < 50.

As in the study with the higher test concentration (100 mg/L) analytical monitoring was performed, this study is selected as key study.

5.4.4 Other aquatic organisms (including sediment)

Table 30: Toxicity to sediment dwelling organisms

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results [µg a.i./L]		Remarks	Reference
			design	duration	EC_{10}	EC_{50}		
OECD 219	*Chironomus riparius*	Development, emergence	Static	28 d	2.09 0.87 (recalc. to mean measured conc.)	3.11	Nominal conc. RI: 1	Dorgerloh, M.; Sommer, H. (2001a) CAR: A 7.4.3.4. /02
Based on guidelines by ASTM (1988, 1990) and USEPA (1975, 1982, 1985)	*Chironomus tentans*	Growth, survival	Semi-static	10 d	0.67 (NOEC)	3.17 (LC50)	Mean measured conc. RI: 1	DAR: Gagliano G.G. (1991)*
OECD 219 Test substance: **Major metabolite imidacloprid desnitro**	*Chironomus riparius*	Development, emergence	Static	28 d	27 9.45 mg/L (recalc. To mean measured conc.)	46	Nominal conc.; RI: 1	Dorgerloh, M.; Sommer, H. (2001b) CAR: A 7.4.3.4 /03

*Study evaluation based on Draft Assessment Report for Imidacloprid 2006 (can be obtained via http://dar.efsa.europa.eu/dar-web/provision)

The long-term effect of Imidacloprid technical on *Chironomus riparius* (A 7.4.3.4. /02) was determined in a study conducted in accordance with the 2001 proposal for a new OECD Guideline 219. Larvae of *Chironomus riparius* were exposed in a static system for 28 days to concentrations of 0.35, 0.64, 1.14, 2.06, 3.70, 5.56 and 10.0 µg/L (nominal) in a water-sediment system (spiked water). The sex, time and number of emerged or not fully emerged adults were recorded daily. The most sensitive endpoint was emergence ratio with an EC_{10} of 2.09 µg/L. This value is based on nominal concentrations. Analytical monitoring of the test substance in overlying and pore water for 3 test concentrations showed a significant decrease in test substance concentration over the exposure period. After 7 days between 34 and 48 % and after 28 days between 20.6 and 25 % of nominal concentration was found in the overlying water. The concentration of the test substance in the pore water was almost negligible. As no measurement of Imidacloprid concentration in the sediment was performed, there is no information whether the decrease in test substance concentration was rather due to degradation or adsorption onto the sediment. Therefore, the use of nominal concentrations may significantly underestimate the toxicity of Imidacloprid to *Chironomus*. To consider the decline in test substance concentration, the geometric mean of the measured concentrations for the time 0, day 7 and day 28 for the nominal concentration 2.09 µg/L (equivalent to the EC_{10}) is calculated. This results in a mean measured concentration of 0.87 µg/L.

In addition, the effect of imidacloprid desnitro, the major metabolite found in water sediment studies, on *Chironomus riparius* over 28 days was determined in a laboratory water sediment

system under static test conditions (A 7.4.3.4 /03). The sex, time and number of emerged or not fully emerged adults were recorded daily. At 64 mg/l, the emergence ratio was significantly reduced (6.7 %), whereas emergence was totally suppressed at 105 mg/l. Start of emergence was postponed at 64 mg/l. Based on these findings, an EC_{10} (development rate, males) of 27 mg/l was calculated. Analytical monitoring of the test substance in overlying and pore water for 3 test concentrations showed a significant decrease in test substance concentration over the exposure period. After 7 days between 27 and 45 % of nominal concentration and after 28 days between 11 and 17 % was found in the overlying water. The concentration of the test substance in the porewater was almost negligible. This means that the use of nominal concentrations significantly underestimates the toxicity of imidacloprid to *Chironomus*. To consider this decline in test substance concentration, the geometric mean of the mean recovery for the 3 measured concentrations for the time 0, day 7 and day 28 is calculated. This results in a mean recovery of 35 %. Applying this recovery to the nominal concentration of 27 mg/l results in an EC_{10} of 9.45 mg/l. The result shows that the metabolite imidacloprid desnitro is by orders of magnitude less toxic to *Chironomus* than the parent substance.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Short-term (acute) aquatic hazard

For Imidacloprid acute studies are available for fish, invertebrates and algae. Invertebrates are the most sensitive trophic level and the most sensitive effect value is a **96h-EC_{50} = 0.00102 mg/L** for *Cloen dipterum* for the endpoint immobilisation.

The criterion for classification as H400 "Very toxic to aquatic life" is a $LC_{50} \leq 1$ mg/L. Hence, Imidacloprid fulfils this criterion and has to be classified as H400. Due to an acute toxicity in the range $0.001 < EC_{50} \leq 0.01$ mg/L an M-factor = 100 has to be applied.

Long-term (chronic) aquatic hazard

Imidacloprid is considered to be not rapidly degradable.

As for Imidacloprid adequate chronic toxicity data is available for all three trophic levels the long-term aquatic classification has to be based on chronic toxicity data. The most sensitive trophic level are invertebrates. The most sensitive species is *Caenis horaria* with a **28d-EC_{10} = 0.000024 mg/L** for the endpoint immobilisation.

For not rapidly degradable substances the criterion for classification as H410 "Very toxic to aquatic life with long lasting effects" is EC_{10}/NOEC ≤ 0.1 mg/L. Imidacloprid fulfils this criterion and has to be classified accordingly. Due to a chronic toxicity in the range $0.00001 < NOEC \leq 0.0001$ mg/L an M-factor = 1000 has to be applied.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

According to CLP Imidacloprid has to be classified as:

Aquatic Acute 1; H400, M = 100

Aquatic Chronic 1; H410, M = 1000

Labelling:

Signal word: Warning

Pictogram: GHS 09

Hazard statement: H410 Very toxic to aquatic life with long lasting effects

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Imidacloprid is an insecticide for plant protection and biocidal products. An environmental harmonised classification as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410, with no M-factors can be found in Annex VI of the CLP, which was translated from the classification under the previous legislation (DSD 67/548/EEC).

The DS proposal for environmental classification was based on new information on aquatic toxicity, which confirms the existing hazard categories and adds appropriate M-factors. The key studies are non-guideline using various non-standard freshwater invertebrate species.

Degradation

In a hydrolysis study (Yoshida, 1989) conducted according to US EPA Guideline § 161-1 and in compliance with GLP, imidacloprid was incubated at 25 °C for 30 days in pH 5, 7 and 9 aqueous solutions. Imidacloprid was found to be stable at pH 5 and 7. Slow hydrolysis with a half-life of approximately 1 year occurred at pH 9 (DT_{50} = 2.75 years, calculated by the DS at 12.5 °C). No significant hydrolysis products were determined.

The photodegradation of radio-labelled imidacloprid in water was studied according to three guidelines US EPA § 161-2, "Photo-transformation of Chemicals in Water", UBA, Germany, Nov. 1990, and OECD TG 316. The studies showed that imidacloprid was rapidly photodegraded in water with half-lives < 1 day. Photodegradation involved the formation of up to 15 phototransformation products, among them four that reached levels higher than 10% of the applied radioactivity.

No ready biodegradability studies were performed.

An aerobic mineralisation in surface water study (Stevens *et. al*., 1997), comparable to OECD TG 309, showed that imidacloprid disappears slowly in non-sterile, non-light exposed test system with a DT_{50} of 331 days at 22°C and a mineralisation rate of 4.3% after 366 d. Imidacloprid was metabolised into nine quantifiable degradation products, among them NTN33893-desnitro, which exceeded 10% of the initially applied radioactivity. No information is available as to whether the degradation products are hazardous to the aquatic environment.

The aerobic transformation of radiolabelled imidacloprid was investigated in two water/sediment studies, conducted according to US EPA § 162-4 and in compliance with GLP. In the first study (Wilmes, 1990), the dissipation behaviour of imidacloprid applied at a concentration of 0.2 mg/L for a 10 cm deep water body was studied in two Dutch water-sediment systems in the dark at 22 ± 1 °C over a period of 92 days. The half-lives (whole system) for the dissipation of imidacloprid calculated according to first-order kinetics were found to be 32 and 142 days for the two systems. CO_2 was formed in both test systems in small quantities (1.4 and 2.0% of the applied radioactivity). Three metabolites were detected in the water phase and the sediment in both test systems. In one system, none of them reached a level of >10% of AR. In the other one, one metabolite (NTN33893-desnitro) reached

a level of 12.3% (sum of amounts found in water and sediment) at the end of the study.

A third water-sediment system, originating from the USA (Stilwell, Kansas) was investigated under aerobic conditions in the dark at 22 ± 1 °C over a period of 30 days (Spiteller, 1993). A first order half-life of 129 days was calculated at 20 °C for the whole system. Negligible mineralisation occurred since 0.7% of the applied radioactivity had been completely mineralised at the end of study. Four metabolites were identified as minor metabolites but none reached a level of 10% of applied radioactivity.

Aerobic degradation in soil was investigated in five laboratory studies with European soils at 20 °C in the dark. First-order half-lives varied between 106 days and 193 days. Mineralisation was limited, accounting for a maximum of 20.3% in one sandy loam soil after 126 days. In total, nine different degradation products have been identified, none exceeding 5%.

In conclusion, the DS considered imidacloprid to be not rapidly degradable as it is hydrolytically stable and not ultimately degraded to a level greater than 70% over 28 days in surface water, water/sediment and soil simulation studies.

Bioaccumulation

Based on experimental data, imidacloprid has a measured log K_{OW} of 0.57 (OECD TG 107, 21 °C and pH 7).

A study on the bioaccumulation behaviour of imidacloprid is not available. The BCF for fish has been predicted from the linear relationship between K_{OW} and BCF developed by Veith *et al*. (1979). According to the "Technical Guidance Document on Risk Assessment Part III", relevant under the Biocidal Products Directive (BPD 98/8/EG), the linear model generated by Veith *et al*., (1979) (log BCF_{fish} = 0.85log K_{OW} - 0.70) can be used for substances with a log K_{OW} < 6. Therefore the calculated value of the bioconcentration factor for imidacloprid in fish on a wet weight basis is BCF_{fish} = 0.609 L.$Kg_{wetfish}$.

Based on a measured log k_{OW} of 0.57 being below the CLP criterion of 4, the DS considered imidacloprid to have a low potential for bioaccumulation.

Aquatic Toxicity

Studies on acute and long-term aquatic toxicity to imidacloprid for all three trophic levels are available. Studies are also available for the main metabolite (NTN33893-desnitro).

The test results are summarised in the following table. The key tests forming the basis for classification are reported in bold.

Table: *Summary of information most relevant for classification on aquatic toxicity*

Method	**Test organism**	**Test system**	**Results**			**Test concentration**	**Reference**
			Endpoint	**LC_{50}/EC_{50} [mg/L]**	**NOEC [mg/L]**		
Fish							
OECD TG 203	*Oncorhynchus mykiss*	Static 96h	Mortality	211		Nominal (confirmed by analytical monitoring)	Anonymous, 1988
U.S.-EPA-FIFRA, 40 CFR, Section	*Oncorhynchus mykiss*	Static 96h	Mortality	> 83		Mean measured	Anonymous, 1990

158.145, Guideline 72-1							
EEC DIRECTIVE 79/831/WG, Annex V	*Leuciscus idus*	Static 96h	Mortality	237		Nominal (confirmed by analytical monitoring)	Anonymous, 1987
OECD TG 210	*Oncorhynchus mykiss*	Flow-through 91d	Time to hatch and swim up		9.02	Mean measured	Anonymous, 2002
Aquatic invertebrates							
Non guideline study	***Cloeon dipterum***	**Static, 96h**	**Immobilisation**	**0.00102**		**Nominal concentrations (confirmed by analytical monitoring)**	**Roessink *et al*., 2013**
Non guideline study	*Cloeon dipterum*	Static, 96h	Immobilisation	0.018		Same experimental setup and species as Roessink *et al.* 2013, but instead of summer generations, winter generations were tested	Van den Brink *et al*., 2016
Non guideline study	*Caenis horaria*	Static, 96h	Immobilisation	0.00177		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Caenis horaria*	Static, 96h	Immobilisation	0.0060		Same experimental setup and species as Roessink *et al.* 2013, but instead of summer generations, winter generations were tested.	Van den Brink *et al*., 2016
Non guideline study	*Plea minutissima*	Static, 96h	Immobilisation	0.0359		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
OECD TG 202	*Daphnia magna*	Static, 48h	Immobilisation	85		Mean measured	Young and Hicks, 1990
Non guideline study	*Notonecta spp.*	Static, 96h	Immobilisation	0.0182		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Limnephilidae*	Static, 96h	Immobilisation	0.00179		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013

Non guideline study	*Asellus aquaticus*	Static, 96h	Immobilisation	0.119		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Chaoborus obscuripes*	Static, 96h	Immobilisation	0.284		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Sialis lutaria*	Static, 96h	Immobilisation	0.0506		Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
OECD TG 202	*Chironomus riparius*	Static, 24h	Mortality	0.055		Nominal (confirmed by analytical monitoring)	Dorgerloh and Sommer, 2002a
US EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	*Hyalella azteca*	Static, 96h	Mortality immobilty	0.526 0.055		Mean measured	England and Bucksath, 1991
US EPA FIFRA, 40 CFR, Part 158.145 Guideline No. 72-2	*Mysidopsis bahia*	Flow-through, 96h	Mortality	0.034		Mean measured	Ward, 1990
Non guideline study	*Cloeon dipterum*	Semi-static 28d	Immobilisation		0.000033 (EC_{10})	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	**Caenis horaria**	**Semi-static 28d**	**Immobilisation**		**0.000024 (EC_{10})**	**Nominal (confirmed by analytical monitoring)**	**Roessink *et al*., 2013**
Non guideline study	*Cloeon dipterum*	Semi-static 28d	Immobilisation		0.4 (EC_{10})	Same experimental setup and species as Roessink *et al.* 2013, but instead of summer generations, winter generations were tested	Van den Brink *et al*. 2016
Non guideline study	*Asellus aquaticus*	Semi-static 28d	Immobilisation		0.00171	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Gammarus pulex*	Semi-static 28d	Immobilisation		0.00295	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Chaoborus obscuripes*	Semi-static 28d	Immobilisation		0.00457	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013

Non guideline study	*Sialis lutaria*	Semi-static 28d	Immobilisation		0.00128	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
Non guideline study	*Plea minutissima*	Semi-static 28d	Immobilisation		0.00203 0.00645	Nominal (confirmed by analytical monitoring)	Roessink *et al*., 2013
US EPA-FIFRA 72-4	*Daphnia magna*	Semi-static 21d	Reproduction, survival, length		1.8 (length)	Mean measured	Young and Blakemore, 1990
OECD TG 202 Test substance: **Major metabolite imidaclopri d desnitro**	*Hyalella azteca*	Static 96h	Mortality	51.8 (LC_{50}) 29.8 (EC_{50})		Mean measured concentration	Roney and Bowers, 1996
Algae and aquatic plants							
OECD TG 201	*Scenedesmus subspicatus*	Static 96h	Growth rate	>10	≥10	Nominal Limit test with 10 mg/L	Heimbach, 1986a
OECD TG 201	*Selenastrum capricornutum*	Static 72h	Growth rate	>100	<100	Nominal (Confirmed by analytical monitoring) Limit test with 100 mg/L	Dorgerloh, 2000
Other aquatic organisms (including sediment)							
OECD TG 219	*Chironomus riparius*	Static 28d	Development, Emergence	0.00311	0.00209 0.00087	Nominal Mean measured	Dorgerloh and Sommer, 2001a
Based on guidelines by ASTM (1988, 1990) and USEPA (1975, 1982, 1985)	*Chironomus tentans*	Semi-static 10d	Growth, survival	0.00317	0.00067	Mean measured	Gagliano, 1991
OECD TG 219 Major metabolite **imidaclopri d desnitro**	*Chironomus riparius*	Static 28d	Development, Emergence	0.046	0.027 0.00945	Nominal Mean measured	Dorgerloh and Sommer, 2001b

Acute toxicity

Three acute toxicity studies to fish are available and included in the CLH Report. In the reliable study by Anonymous (1988), the short-term toxicity of imidacloprid (technical active substance) was examined on young rainbow trout (*Oncorhynchus mykiss*) under static condition and according to OECD TG 203. A 96h LC_{50} of 211 mg/L based on nominal

concentrations (analytically confirmed - measured > 80% of nominal concentrations) was determined. This study is considered as acceptable with fulfilled validity criteria and used as relevant data for purpose of the acute classification.

Further short term fish toxicity studies Anonymous (1990) and Anonymous (1987), conducted respectively with *Oncorhynchus mykiss* and *Leuciscus idus* according to EPA Guideline 72-1 and EEC directive 79/831/WG-1984, are reported as adequate acute toxicity data and used as supplementary information.

Based on the available data, imidacloprid shows a low acute toxicity to fish with a reliable LC_{50} value >1 mg/L.

Short-term toxicity tests with 10 aquatic invertebrate species from different taxonomic groups are available (Roessink *et al*. 2013). Test organisms were collected from an uncontaminated aquatic ecosystems. Early larval insect instars were used for the tests. The test organisms were acclimated for at least 3 days to laboratory conditions (18 +/- 2 °C, 12:12 hours light: dark). The exposure period was 96h and the endpoints used were immobilisation and mortality. Imidacloprid concentrations measured in the dosing solution were, on average, 97.5% of the nominal concentration. No further analytical monitoring was performed. However, from the analytical monitoring performed for the long-term studies the DS concluded that the test substance concentration was stable during the exposure period of 96h and thus the use of nominal concentrations is justified. Concerning the validity criteria of OECD TG 202 (*Daphnia* Acute Immobilisation Test), the criterion of 10% maximum immobilisation in controls is fulfilled for 8 of the 10 tests. In the summarising table above, only studies with reliability Klimisch score 1 and 2 were reported. The 96h EC_{50} values range from 1.02 – 284 µg/L for the endpoint immobilisation. The most sensitive species were *Cloeon dipterum* (1.02 µg/L), *Caenis horaria* (1.77 µg/L) and *Limnephilidae* (1.79 µg/L).

The DS also provided another non guideline study (van den Brink *et al*. 2016) with the same experimental setup and *Ephemeroptera* species as Roessink *et al*. (2013) but instead of summer generations, winter generations were tested. The short-term toxicity values for the winter generations are higher than for the summer generations. Therefore, the DS proposed to use for classification the lowest toxicity values for the summer generations, as most relevant for hazard assessment.

No effects were seen in two limit tests with **green algae** at the concentration of 10 mg/L and 100 mg/L.

Based on the 96h EC_{50} (immobilisation) of 0.00102 mg/L for *Cloeon dipterum*, the DS proposed classification as Aquatic Acute 1 (M=100).

Chronic toxicity

A single chronic toxicity study on fish performed with Imidacloprid is provided in the CLH Report. In this study (Anonymous, 2002), the long term toxicity of Imidacloprid (technical active substance) was tested on *Oncorhynchus mykiss* in a fish early life-stage study conducted according to OECD TG 210. Observed endpoints were time to hatch and hatching rate, larval deformities and survival, time to swim-up, behavioural changes and post-hatch survival and growth. Based on mean measured concentrations, the NOEC was determined to be 9.02 mg/L for the most sensitive endpoints (time to hatch and swim up). For the other observed endpoints the NOEC was 26.9 mg/L. This study is considered valid and useful for purpose of chronic classification.

Based on the available data, Imidacloprid shows a low chronic toxicity to fish, with a lowest

NOEC of 9.02 mg/L.

Regarding aquatic invertebrates, long-term toxicity tests with species from 7 different taxonomic groups were available (Roessink *et al*., 2013). Test organisms were collected from an uncontaminated aquatic ecosystem. Early larval insect instars were used for the tests, after acclimation for at least 3 days to laboratory conditions (18 ± 2 °C, 12:12 hours light: dark). Five concentrations and a control were tested using 3 replicates with each 10 test animals. Immobilisation and mortality were the detected endpoints for an exposure period of 28 d. Every week the test solution was renewed. Imidacloprid concentrations measured in the dosing solution were, on average, 95.5% of the nominal concentration. Analytical monitoring was performed for the control and the highest test concentration. Measured concentrations were in the range of 84.9 – 97% of the nominal concentration, thus proving the test substance to be stable during the exposure phase. 28d EC_{10} values (immobilisation) for the 7 tested species were in the range of 0.024 – 4.57 µg/L.

As in the short-term studies, the mayflies *Cloeon dipterum* (28d EC_{10} = 0.033 µg/L) and *Caenis horaria* (28d EC_{10} = 0.024 µg/L) were most sensitive.

The DS provided also the result from non-guideline study by Van den Brink *et al*. (2016) with *Cloeon dipterum*. The experimental setup was the same as Roessink *et al*. (2013) but instead of summer generations, winter generations were tested. The 28d EC_{10} value (immobilisation) was 0.40 µg/L. The values for the winter generations are higher than for the summer generations. Therefore, the DS proposed to use for classification the lowest toxicity values for the summer generations, as most relevant for hazard assessment.

In one limit test with green alga *Scenedesmus subspicatus*, no effects were seen up to and including the highest dose tested, 10 mg/L. However no analytical monitoring was performed, so the effect value was based on nominal concentrations. In another limit study with *Selenastrum capricornutum*, the limit dose of 100 mg/L did have a statistically significant effect on growth rate, but this effect was < 50%. Therefore, NOE_rC value was <100 mg/L.

Furthermore, the toxicity studies on the metabolite imidacloprid desnitro to *Hyalella azteca* (OECD TG 202) and to *Chironomus riparius* (OECD TG 219) showed that it is less toxic compared to parent compound.

Based on the 28d EC_{10} (immobilisation) of 0.00024 mg/L for *Caenis horaria*, the DS proposed classification as Aquatic Chronic 1 (M=1000).

In summary, the DS considered Imidacloprid as not rapidly degradable but not potentially bioaccumulative for classification purposes. The selected acute toxicity EC_{50} value is between 0.001-0.01 mg/L resulting a classification of Aquatic Acute 1 with M-factors of 100. The selected chronic toxicity EC_{10} value is between 0.00001-0.0001 mg/L, resulting a classification of Aquatic Chronic 1 and M=1000.

Comments received during public consultation

Four MSCAs commented the proposal and expressed a general agreement with the proposed classification based on mayflies. However, a commenting Company-Manufacturer raised several doubts on the non-standard species used to classify the substance. Most of the MSCAs also highlighted the need for more detailed information on the non-guideline key studies in order to verify the validity criteria and the actual concentrations of Imidacloprid used in the tests.

The well-argued responses by the DS are reported in the "additional key elements" section in the Background Document.

Additional key elements

The following information is provided by the DS in response to PC comments, reported from Roessink *et al*. (2013) and van den Brink (2016):

Test item: SL formulation containing 200 g/L imidacloprid. In a subsequent publication (van den Brink, 2016) some tests were also performed with technical grade imidacloprid (purity not given). For *Caenis horaria* (overwintering generation) a 96h EC_{50} of 6 µg/L is reported, that supports the EC_{50} of 1.77 µg/L and would result in the same M-factor. Therefore, the DS concluded that the use of the SL formulation instead of pure imidacloprid would not influence the test results.

Test item media: Copper-free water, 1.5 L jars with 1 L copper-free media. Stainless steel meshes were introduced to the test system to serve as substrate.

Exposure treatment preparation: Immediately after the animals were transferred into the test jars containing test water, an appropriate volume of imidacloprid stock solution was spiked using a capilettor.

48h effect values: This information is not available from Roessink *et al.* (2013). However, from a subsequent publication (van den Brink *et al*., 2016) supplemental data are available also for the experiments by Roessink *et al.* (2013). The following data are given for *Cloeon dipterum*:

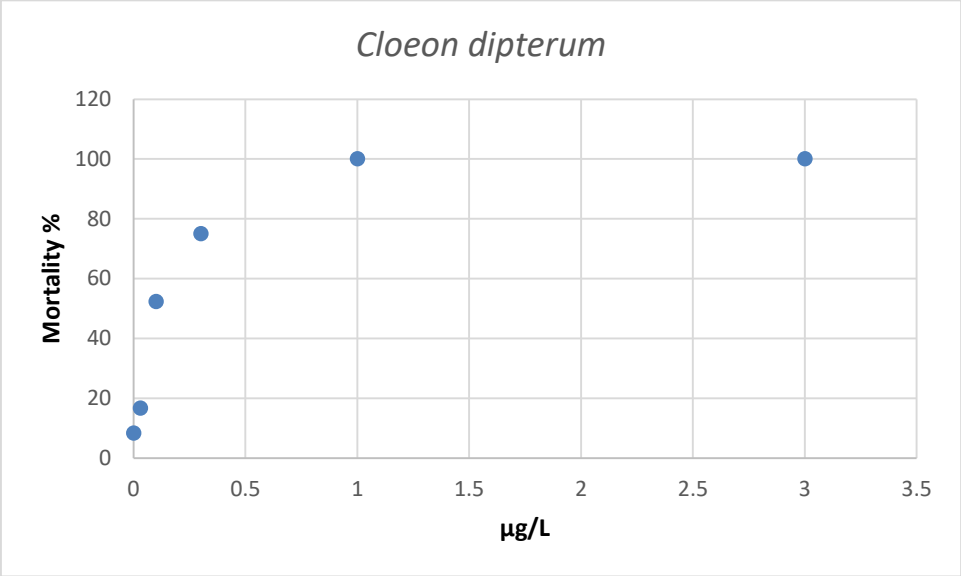
Exposure time	EC_{50} [µg/L]
24h	72
48h	2.7
72h	1.7
96h	1.0

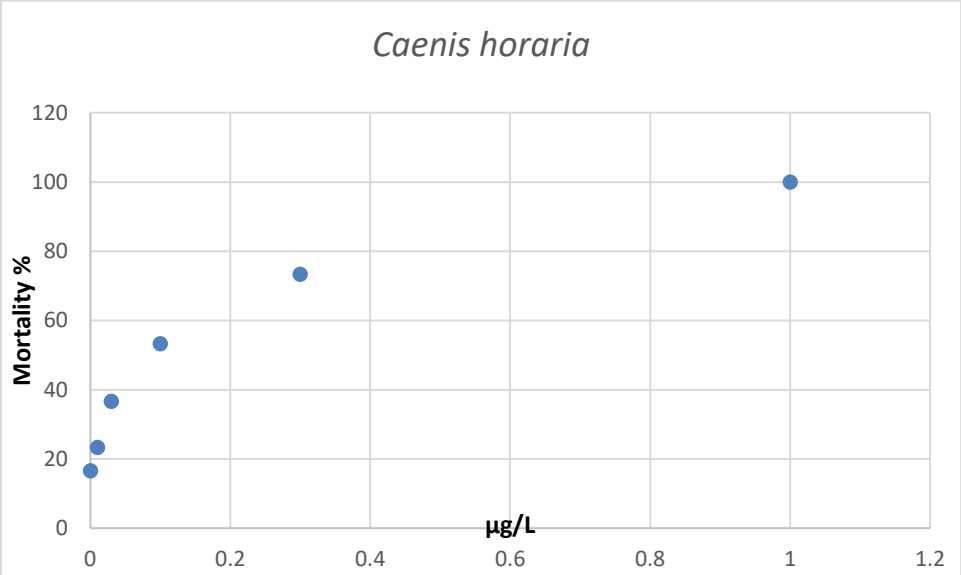
These data indicate that even if the standard test duration of 48h is considered, the classification proposal would not change.

Acute and chronic raw data: Raw data for the acute tests are not available. Raw data for the chronic tests (confidential) indicate that in the 28d test with *Caenis horaria* no emergence of test animals occurred. In the 28d test with *Cloeon dipterum*, the number of emerged animals was between 0 (highest test concentration with 100% immobility) and 6 (in one replicate of the lowest test concentration). Average emergence over all replicates and test concentrations was 2. Emerged animals were counted as missing in the statistical analysis.

Concerning the difference between the replicates in the chronic tests, for both *Cloeon dipterum* and *Caenis horaria* the replicates of the single test concentrations are in good agreement.

Acute and chronic toxicity dose-response curves: Dose-response curves are not given in the publication. For the acute tests, the slope of the dose-response function is given with 1.29 for *Caenis horaria* and 0.944 for *Cloeon dipterum*. For the chronic studies, the slope is given as 1.32 for *Caenis* and 1.67 for *Cloeon*. From the raw data available for the chronic studies, the following dose-response curves were derived by the DS:





Test organisms: Taxonomic identity of the organisms used in the study are sufficiently described including genus and species. Origin of test organism is also sufficiently described.

Test setup: Test system is adequately described in terms of testing procedure, origin and acclimation of the test organisms, test conditions (temperature, dissolved oxygen, pH, lighting), test duration, type of exposure, test concentrations, number of replicates, number of test organisms per replicate, analytical monitoring of test substance concentration, control mortality/immobilization, statistical method for derivation of effect values.

In addition the validity criteria of OECD TG 202 (*Daphnia* acute test) were fulfilled for the acute tests (control mortality not above 10%, oxygen content ≥ 3 mg/L) and validity criteria of OECD TG 211 (Daphnia reproduction test) (control mortality not above 20%) was fulfilled for the chronic tests.

Considering the available information, the DS considered it appropriate to give the study a reliability score of 2.

Moreover, RAC noted that, as the DS explained, supplemental data are available from a subsequent publication by Van den Brink *et al*. (2016) in table above, referring also to the experiments by Roessink *et al*. (2013). The following data are given for *Cloeon dipterum* in table S4 (Supplemental Information) by Van den Brink *et al*. (2016).

Test 2: Acute test with imidacloprid (May/June 2012) and Tab.3, Roessink *et al*., 2013	
Exposure time	EC_{50} [µg/L]
24 h	72
48 h	2.7
72 h	1.7
96 h	1.0
Test 10: Chronic test with imidacloprid (Aug/Sept 2012) and Tab.4, Roessink *et al*., 2013	
Exposure time	EC_{10} [µg/L]
7 d	0.001
14 d	0.004
21 d	0.056
28 d	0.024

The 48h and 21d results are both available, and can be used for classification purpose. However, the conclusions for the acute and chronic classification are not affected.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider imidacloprid as not rapidly degradable. The substance is hydrolytically stable and not ultimately degraded to a level greater than 70% over 28 days in surface water, water/sediment and soil simulation studies.

Bioaccumulation

As experimentally determined BCF values are not available for imidacloprid, the assessment of bioaccumulation is based on experimentally determined log K_{OW} value. Hence, based on the value of log K_{OW} = 0.57 being below the decisive CLP criterion (log K_{OW} < 4), RAC agrees with the DS proposal to consider the bioaccumulation potential of imidacloprid as low.

Aquatic toxicity

Invertebrates are the most sensitive trophic level. The key study is non guideline and performed with non standard species, i.e. different species of mayflies. Insects have to be considered a representative group for the invertebrate trophic level, as the mode of action of Imidacloprid implies acting as antagonist of the nicotinic acetylcholine receptor in the central nervous system of insects, thus disturbing synaptic signal transmissions of insects as Mayflies. Consequently, RAC considers the study relevant as well as reliable for use in classification.

Acute aquatic hazard

Acute aquatic toxicity studies are available for fish, invertebrates and algae. The most sensitive species were *Cloeon dipterum* and *Caenis horaria* in the same range of sensitivity. The key study is performed with non-standard different species of mayflies. Nevertheless, RAC considers the study relevant and reliable for use in classification. RAC concludes that, in order

to provide consistency with the results from OECD TG 202, the 48h results can be used for classification. This does not change the proposal from that of the DS.

In conclusion, the most sensitive effect value is for *Cloen dipterum* (immobilisation). With a 48h EC_{50} = 0.0027 mg/L, imidacloprid meets the classification as Aquatic Acute 1, M-factor=100, because the acute toxicity value is in the range 0.001 < EC_{50} ≤ 0.01 mg/L.

Chronic aquatic hazard

Adequate chronic toxicity data is available for all three trophic levels. As for the acute toxicity, invertebrates are the most sensitive group. The lowest value is for mayflies with a 21d EC_{10} = 0.000056 mg/L (immobilisation). The key study is performed with a different non-standard species, nevertheless RAC considers the study relevant and reliable for use in classification. Similarly to the aquatic acute classification, RAC concludes that, in order to provide consistency with the results from OECD TG 211, the available 21d results can be used for classification. This does not change the proposal from that of the DS.

Imidacloprid fulfils the criteria for classification as Aquatic Chronic 1, M-factor =1000, because the chronic toxicity value is in the range of 0.00001 < NOEC ≤ 0.0001 mg/L and is considered not rapidly degradable.

In conclusion, RAC agrees with the DS that imidacloprid warrants classification as Aquatic Acute 1 (M=100) and Aquatic Chronic 1 (M=1000).

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7 ANNEXES

Confidential Annex to the CLH Report