Committee for Risk Assessment

RAC

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

Adopted

13 June 2019

13 June 2019

CLH-O-0000001412-86-282/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **428-040-8**

CAS Number: **138261-41-3**

The proposal was submitted by **Germany** and received by RAC on **3 September 2018.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 October 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 December 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Annemarie Losert**

Co-Rapporteur, appointed by RAC: **Pietro Paris**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **13 June 2019** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	612-252-00-4	imidacloprid (ISO); *(E)*-1-(6-chloropyridin-3-ylmethyl)-N-nitroimidazolidin-2-ylidenamine	-	138261-41-3	Acute Tox. 4 * Aquatic Acute 1 Aquatic Chronic 1	H302 H400 H410	GHS07 GHS09 Wng	H302 H410			
Dossier submitters proposal	612-252-00-4	imidacloprid (ISO); *(E)*-1-(6-chloropyridin-3-ylmethyl)-N-nitroimidazolidin-2-ylidenamine	-	138261-41-3	**Modify** Acute Tox. 3 **Retain** Aquatic Acute 1 Aquatic Chronic 1	**Modify** H301 **Retain** H400 H410	**Modify** GHS06 Dgr **Retain** GHS09	**Modify** H301 **Retain** H410		**Add** Oral: ATE = 131 mg/kg bw M=100 M=1000	
RAC opinion	612-252-00-4	imidacloprid (ISO); *(E)*-1-(6-chloropyridin-3-ylmethyl)-N-nitroimidazolidin-2-ylidenamine	-	138261-41-3	Acute Tox. 3 Aquatic Acute 1 Aquatic Chronic 1	H301 H400 H410	GHS06 GHS09 Dgr	H301 H410		Oral: ATE = 131 mg/kg bw M=100 M=1000	
Resulting Annex VI entry if agreed by COM	612-252-00-4	imidacloprid (ISO); *(E)*-1-(6-chloropyridin-3-ylmethyl)-N-nitroimidazolidin-2-ylidenamine	-	138261-41-3	Acute Tox. 3 Aquatic Acute 1 Aquatic Chronic 1	H301 H400 H410	GHS06 GHS09 Dgr	H301 H410		Oral: ATE = 131 mg/kg bw M=100 M=1000	

GROUNDS FOR ADOPTION OF THE OPINION

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.

HUMAN HEALTH HAZARD EVALUATION

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	LD50: 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	LD50: 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

Guideline Route, Species GLP	Species, Strain Sex No of animals	Dose levels Frequency of application	Results	Remarks	References
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.

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.

ENVIRONMENTAL HAZARD EVALUATION

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

Method	Test organism	Test system	Results			Test concentration	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
U.S.-EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-1	*Oncorhynchus mykiss*	Static 96h	Mortality	> 83		Mean measured	Anonymous, 1990
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

Method	Test organism	Test system	Results			Test concentration	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
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

Method	Test organism	Test system	Results			Test concentration	Reference
			Endpoint	LC_{50}/EC_{50} [mg/L]	NOEC [mg/L]		
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 imidacloprid 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 **imidacloprid 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.

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).

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).