ACES REPORT 27

Imidacloprid

Data evaluation

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Preface

The Department of Environmental Science and Analytical Chemistry (ACES) at Stockholm University was commissioned, by the Swedish Agency for Marine and Water Management, to evaluate an ecotoxicity study (Roessink et al. 2013) for the adoption of an EQS for the specific pollutant Imidacloprid.

The evaluation was prepared by Sara Sahlin and Marlene Ågerstrand.

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Förtydligande från Havs- och vattenmyndighetens

Havs- och vattenmyndigheten planerar att ta med imidakloprid bland de ämnen som regleras i Havs- och vattenmyndighetens föreskrifter (HVMFS 2013:19) om klassificering och miljökvalitetsnormer avseende ytvatten[1]. Imidakloprid har tidigare bedömts inom olika lagstiftningar (biocidlagstiftning, växtskyddsmedelslagstiftning och holländskt värde enligt vattendirektivet) men genererat tre olika värden för limnisk miljö beroende på metodval.

Stockholms Universitet har på uppdrag av Havs- och vattenmyndigheten kvalitetsgranskat den mest kritiska studien i beslutsunderlaget. Denna studie (Roessink et al. 2013) bedömdes som tillförlitlig och kan därmed beaktas vid etablering av bedömningsgrund.

Notera att ämnet står med på bevakningslistan. Ett EU-gemensamt värde för tillämpning inom vattenförvaltningen kan komma att tas fram.

[1] https://www.havochvatten.se/hav/vagledning--lagar/foreskrifter/register-vattenforvaltning/klassificering-och-miljokvalitetsnormer-avseende-ytvatten-hvmfs-201319.html

1. ROESSINK ET AL. (2013)

Roessink et al. (2013) conducted short and long-term ecotoxicity tests using 10 and 7 aquatic arthropods, respectively. The test organisms represented a range of taxonomic orders of which the species *Cloeon dipterum* and *Caenis horaria* (mayflies - *Ephemeroptera*) showed greatest sensitivity with EC_{10}[2] (0.033 and 0.024 μg/L) substantially lower compared to other species tested. The effect values reported in this study have been included when deriving regulatory acceptable concentrations for freshwater organisms within three different regulatory frameworks (table 1). RIVM (2016) and EFSA (2014a) derived threshold concentrations based on SSD's which included data for crustacean and insects. In all cases, a lower assessment factor (AF) was applied since the data with the most sensitive species were included.

Table 1. Regulatory acceptable concentrations derived for three different regulatory frameworks.

Reference and legislation	Reliability Evaluation: Rosseink et al. (2013)	Method and AF	Value derived
RIVM, 2014 Water framework Directive	Reliable with restrictions (R2)	SSD, AF 3	8.3 ng/L (AA-EQS)
CAR, 2015 Biocidal product Directive	Reliable with restrictions (R2)	AF method, AF 5	4.8 ng/L (PNEC)
EFSA, 2014a (Tier- 2B) Plant protection legislation	Not considered fully reliable[1]	SSD, AF 3	9 ng/L (RAC)[2]

1 = The study was not assigned a reliability score in EFSA (2014a). 2 = Provisional value due to limitations related to the dataset.

During the EFSA expert consultation meetings (EFSA, 2014b) two main issues were discussed regarding the Roessink et al. (2013) study: (i) the lack of information to assess the reliability, and (ii) that the acute endpoints were inconsistent with a similar study.

(i) Initially, the general view of the experts were that the study was reliable. However, EFSA and some of the experts did not considered it possible to, in detail, assess the study due to lack of information such as raw data, sampling period of the organisms, and analysis of the tested concentration. It was agreed that the study should be considered reliable pending the evaluation of the requested information, and that the endpoints could be used for risk assessment in a conservative approach.

[2] Note that the species names in table 4 in Roessink et al. (2013) are incorrect. The chronic effect values reported for *C. horaria* actually referees to *D. dipterum*.

Later, EFSA was provided with raw data. The authors stated that the organisms used for chronic testing was sampled in August[3] and that the results referred to the active substance. However, EFSA noted that there were some inconsistencies regarding the raw data and the information in the study (e.g. the control mortality slightly differed). The DE expert(s) argued that although correcting the control mortality, it was still within the range of acceptable control mortality (12%). Furthermore, EFSA noted that the exposure data were not consistent with the tested concentrations reported in the article. Once again DE argued that this could easily be solved by clarification from the authors.

(ii) An additional acute study (indoor, single-species study, GLP) of *C. horaria* and *C. dipterum* with similar test-set up has been discussed (van Wijngaarden and Roessink 2013). The reported 96h EC_{50} were more than 10 times higher compared to those in Roessink et al. (2013). Therefore, EFSA and the experts requested argumentation/explanation of the differences in the result. The experts noted that the test organisms were collected in October, which could explain the disperse results by seasonal variability of the species (reduced sensitivity of winter generations). However, EFSA noted that both studies used organisms at nymph stage and that it was unlikely that nymphs collected in October belonged only to the winter generation (since summer generations may mate until the end of August)[2]. It was stated that the potential difference in sensitivity between the two seasonal generations might not fully explain the inconsistent result. NL, DK and DE, however, argued that the study was reliable and supported the inclusion of the results in the derivation.

In a third study (outdoor, mesocosms study) by Roessink and Hartgers (2014)[4] it was noted that temperature may influence the sensitivity of *C. dipterum*. The study generated a factor of 46 lower toxicity to *C. dipterum* and was conducted at lower temperature. In the study evaluation from RIVM it was concluded that the study does not represent a worst-case scenario since it was conducted during the autumn.

New available information: van den Brink et al. (2016), co-authored by Roessink, showed once again that the winter generation (of *C. dipterum* and three other species) exhibited lower toxicity compared to organisms collected in April (table 2). In addition, temperature only slightly affected (factor of 1.7) the sensitivity when increasing the temperature from 10°C to 18°C (results not reported in table 2).

[3] In a recent publication co-authored by Roessink, it is stated that the organisms for acute toxicity testing were collected in May-June (van den Brink et al. 2016).

[4] This study was not considered in EFSA's derivation, it was only included as additional information.

Table 2. Effect values (µg/L) for *C. dipterum* reported in van den Brink et al. (2016), Roessink et al. (2013), and van Wijngaarden and Roessink (2013).

Period when organisms were collected	Immobilization		Mortality		Reference
	EC10	EC50	LC10	LC50	
Acute 96h					
May/June 2012	0.10	1.02	6.16	26	Roessink et al. 2013
April 2013	21	25	15	34	Van den Brink et al. 2016
October 2012	-	12	-	-	van Wijngaarden and Roessink 2013
December 2013	11	18	11	37	Van den Brink et al. 2016
Chronic 28d					
August/September 2012	0.024	0.13	0.24	0.32	Roessink et al. 2013
November/December 2013	0.40	0.68	0.65	0.85	Van den Brink et al. 2016

2. EVALUATION OF ROESSINK ET AL. (2013)

Assessing immobility

Paralysis effects of crustaceans through imidacloprid exposure has been seen at lower concentrations compared to those required to cause death, thus immobilization is considered as a relevant endpoint (Sánchez-Bayo and Goka 2006). The definition of immobility and death in the current study was as followed:

"*Scored as immobile when no movement of any kind was observed for a period of 20 s and were scored as dead when no response of any kind was observed during 3 to 5 s of gentle stimulation using a Pasteur's capillary pipette*".

While OECD guidelines define immobility and/or death as:

"Those animals that are not able to change their position (by crawling or swimming movements) within 15 seconds after mechanical stimulation, e.g. by subjecting the larvae to a gentle stream of water from a Pasteur pipette or agitation of the test vessel, are considered to be immobilized" (OECD guideline 235 - acute immobilization test for *C. Riparius*).

"Those animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel are considered to be immobilized (even if they can still move their antennae)" (OECD guideline 202 - Daphnia sp. acute immobilization test).

"An animal is recorded as dead when it is immobile, i.e. when it is not able to swim, or if there is no observed movement of appendages or postabdomen, within 15 seconds after gentle agitation of the test container. (If another definition is used, this must be reported together with its reference)" (OECD guideline 211 – Daphnia magna reproduction test).

Commonly, the guidelines definition of immobility refers to lack of movement after mechanical stimulation (usually after 15 seconds). In Roessink et al. (2013) organisms scored as immobile were not

subject of any mechanical stimuli (neither to the organisms nor the vessel). However, this issue was not discussed during the EFSA expert consultation meetings (EFSA, 2014b). The same methodology was used to determine immobilization in van den Brink et al. (2016), thus, how immobility was defined could not explain the dispersed results.

Reliability evaluation

The evaluation of the reliability was performed using the CRED evaluation method (Moermond et al. 2017). The overall conclusion of the reliability evaluation (table 3) is that the study is reliable with restrictions (R2)[5]. Based on EFSA's argument it is not certain if the organisms belonged to the summer generation (since field-collected in August). It remains unclear if it is reasonable that the seasonal variation could explain the differences of the results in van den Brink et al. (2016) and Roessink et al. (2013), of which the acute EC_{50} differs approximately by a factor of 25 and the chronic EC_{10} by a factor of 16 (table 2). An additional study using the spring/summer generation is necessary to clarify the actual seasonal variation. Meantime, there seem to be no clear reason for rejecting Roessink et al. (2013).

[5] The study was assessed as relevant without restrictions (C1) (CRED evaluation not reported).

Table 3. CRED reliability evaluation of Roessink et al. 2013.

Species	*Cloeon dipterum* (early larvae instar) (most sensitive species of all tested)		
Endpoint	Mortality and immobilization		
Effect value (µg/L)	EC_{10} = 0.024 (Cl: 0.006-0.091) for immobilization. LC_{10} = 0.235 (Confidence interval could not be calculated) for mortality		
Criteria	**Fulfilment**	**Information**	**Comment**
Is the guideline method (OECD/ISO) or modified guideline used?	Not fulfilled		Non- guideline study
Is the test performed under GLP conditions?	Not fulfilled		
If applicable, are validity criteria fulfilled (e.g., control survival, growth)?	Partly fulfilled	28d control mortality 13 %; control immobilisation 17% 48h control mortality 10%; control immobilisation 10%. .	Immobilization is usually determined in acute test. According to OECD 202, not more than 10% of Daphnids in the control should be immobile after 48h. In OECD 235, 15% of control immobilization is acceptable for *C. riparius.*
Are appropriate controls performed (e.g., solvent control, negative and positive control)?	Fulfilled		
Is the test substance identified with name or CAS number? Are test results reported for the appropriate compound?	Fulfilled	Identified with name, not CAS number. Endpoints expressed as active substance according to EFSA 2014b.	
Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	Partly fulfilled	*"soluble concentrate (SL) formulation containing 200 g imidacloprid/L made up in 2.5- or 7.5-ml dosing aliquots for application using a 2.5-ml capilettor. Samples were taken from the dosing solutions to confirm imidacloprid concentrations"*	

Criteria	Fulfilment	Information	Comment
If a formulation is used or if impurities are present: Do, other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	Unknown	IMIDACLOPRID SL 200 (formulation)	
Are the organisms well described (e.g., scientific name, weight, length, growth, age/life stage, strain/clone, gender if appropriate)?	Partly fulfilled	Scientific name and life stage. Field-collected. Collected in May-June (acute) and in August (chronic) according to Brink et al. (2016) (co-author Roessink). Early larval stage.	
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	Partly fulfilled	Field-collected (from uncontaminated aquatic ecosystems). Acclimatized (18°C and 12:12 hours light:dark) first in a mixture of field and test water, then in only in test water—for at least 3 d..	No information about what type of habitat the larvae was collected from.
Is the experimental system appropriate for the test substance. Taking into account its physicochemical characteristics?	Fulfilled	Static-renewal (weekly renewal); glass-jars	
Is the experimental system appropriate for the test organism (e.g., choice of medium or test water, feeding, water characteristics, temperature, light/dark conditions, pH, oxygen content)? Have conditions been stable during the test?	Expert judgment required to determine	Reconstituted water; water only-exposure; pH (7.36–8.30), DO (7.06–9.59 mg/L), conductivity (179–208 mS/cm), and temperature (17.7–19.78°C). Fed with biofilm, organic matter and periphitic algae.	The nymphs of *C. horara* are borrowed into, and live on mud and silt. Are poor swimmers and adapted for movements of mud and silt where they feed by collecting or gathering fine particulate organic detritus from the sediments (http://www.riverflies.org/caenis-horaria-anglers-curse)

Criteria	Fulfilment	Information	Comment
Were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	Fulfilled	Water solubility 610 mg/L	
Is correct spacing between exposure concentrations applied?	Fulfilled	0.01; 0.03; 0.1; 0.3; 1 (factor of approximately 3)	
Is the exposure duration defined?	Fulfilled	28d	
Are chemical analyses adequate to verify concentrations of the test substance over the duration of the study?	Partly fulfilled	Results based on nominal concentrations. 84,9±4,5% of nominal concentration (time-weighted average measured concentrations in the chronic tests as a percentage of the nominal concentrations. For 4 weeks and 3 replicates). Water samples from the control and highest treatments were collected for residue analysis at the end of each test week. Results of the measurements were then used to calculate time-weighted average exposure concentrations	
Is the biomass loading of the organisms in the test system within the appropriate range (e.g., <1 g/L)?	Fulfilled	10 organisms/ 0,5 L. *"For all species, the same test systems were used as in the acute tests, except for C. horaria, for which we used 0.5-L glass jars rather than 1.5-L glass jars"*	
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?	Partly fulfilled	3 replicates (10 organisms each)	*"Emerged individuals were removed weekly and were counted as missing in the statistical analysis because after emergence, it is no longer possible to determine whether the individual would have been affected."* Not specified how many organisms that were eliminated from the statistical analysis.

Criteria	Fulfilment	Information	Comment
Are appropriate statistical methods used?	Fulfilled	The log-logistic regression was performed using GenStat 15th edition (Laws Agricultural Trust; VSN International). 95% confidence interval.	
Is a concentration–response curve observed? Is the response statistically significant?	Fulfilled	No dose-response curve reported. Slope parameter of the dose-response function =1,32 (immobilization) and 7,43 (mortality). Raw-data has been provided to EFSA, clear dose response (EFSA, 2014b) Effect values (derived from same test) 28d EC10=0.024 (CI 95% 0.006-0.091) 28d LC10=0.235 (CI could not be calculated) 28d EC50= 0.126 (CI 95% 0.070-0.228) 28d LC50=0.316 (CI could not be calculated)	
Are sufficient data available to check the calculation of endpoints and (if applicable) validity criteria (e.g., control data, concentration– response curves)?	Fulfilled	Raw-data was provided to EFSA (in EFSA 2014b)	

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