Bustos et al.
Sustainable Environment Research (2022) 32:30
https://doi.org/10.1186/s42834-022-00141-y

Sustainable Environment Research

RESEARCH

Open Access

Species sensitivity distribution of dichlorvos in surface water species

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Abstract

Dichlorvos is an organophosphorus insecticide frequently detected in surface waters all around the world. From an evaluation of the environmental quality concentrations (EQC) for dichlorvos in surface waters adopted by different countries, it was observed a wide variability among them. This is despite regulatory EQC-values are typically based on toxicity data and species sensitivity distribution (SSD) in all the investigated regulatory frameworks, and therefore should be similar. Hence, what is the cause of the differences between national and regional EQC-values? And, which ones will protect the aquatic fauna? These hypotheses were proposed to explain differences among SSDs based on the choice of toxicity data: (i) EQC values obtained from technical presentation (pure dichlorvos) will be higher than the estimated from dichlorvos formulation (containing other substances to improve the efficiency of the active principle), as they may include synergists; (ii) different taxa will have different sensitivities; (iii) data produced under different experimental conditions will severely affect the SSD. Regarding their capacity to protect the aquatic fauna the hypotheses were; iv) environmental concentration of dichlorvos represents a risk for aquatic organisms; and v) not all EQC-values are protective for the aquatic fauna. These were tested through a meta-analysis of toxicity data enabling the construction of SSD's across technical and formulated dichlorvos and species of several taxa, and across literature and experimental data produced under analogous conditions. Finally, the EQC elaborated were compared with a meta-study on monitored environmental concentrations. The study suggested that technical dichlorvos increased toxicity compared to formulated products up to two-fold for arthropods. Species phylogeny affected sensitivity, but the SSD derived values used for setting regulatory concentrations were remarkably robust to the inclusion/exclusion of less sensitive species. The SSD results from the literature and experimental data were similar in the case of technical dichlorvos results. The regional differences in EQC values therefore most likely stem from political considerations on how to use SSDs to derive EQCs rather than from differences in SSDs. The experimental SSD defined a protective concentration of 6.5 ng L^{-1} for 5% of the species, which is according to the European EQC, but one to two-fold lower than the limit values of the US, China, and Argentina.

Keywords: SSD analysis, Dichlorvos, Surface-water community, Guidelines

1 Introduction

The aquatic environments are under pressure due to human activities where xenobiotics such as pesticides, pharmaceuticals, and industrial chemicals are daily released to surface waters [1]. Pesticides are easily spread in the environment as they may reach water matrices during their application (drift), by run-off and/or by leaching, therefore representing a risk on the preservation of aquatic environments [2]. Adverse effects of pesticides on non-target organisms have been widely reported [3]. Despite the relatively low occurrence and concentration of neuroactive insecticides in natural waters, they have been identified as being of high concern [4] with organophosphates receiving most attention in terms of

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aquatic toxicity. Dichlorvos (2,2-dichloroethyl dimethyl phosphate) is an organophosphate insecticide acting as an acetylcholinesterase enzyme inhibitor [5]. Dichlorvos is mainly used in agriculture, for grain storage, and pest control for livestock and households [6], and has been detected in surface waters from both agricultural and non-agricultural areas [2, 7, 8]. A recent review showed that the monitored mean concentration of dichlorvos ($130\,ng\,L^{-1}$) exceeded the sum of all other monitored organophosphates compounds [2], and exceeded regulatory concentration for water wildlife protection in Argentina, the United States (US), Europe, and China [9–12], hence its occurrence is no doubt a problem.

Comparing the regulatory concentrations for dichlorvos, wide variability among the different regions is observed. For instance, the Environmental Protection Agency of the US (USEPA) set an acute benchmark concentration for dichlorvos of $0.035\,\mu g\,L^{-1}$ and chronic $0.0058\,\mu g\,L^{-1}$ for aquatic invertebrates [10]. However, the European Commission adopted an environmental quality concentration (EQC) for dichlorvos three orders of magnitude lower, defined as a maximum available concentration of $0.0007\,\mu g\,L^{-1}$ [11]. In Argentina, the National Agency of Hydrological Resources established an acute benchmark concentration for dichlorvos of $0.078\,\mu g\,L^{-1}$ and chronic of $0.0078\,\mu g\,L^{-1}$ [9]. A value of $0.10\,\mu g\,L^{-1}$ has been proposed in China based on the SSD approach considering native species [12]. As is observed, there is a remarkably broad range of regulatory concentrations varying up to 100-fold between the highest and lowest values. What is the cause of the differences among the regulatory concentrations? And will they all protect the aquatic fauna?

The cause of the difference in regulatory concentrations could be attributable to several factors such as differences in the method used to derive them [13]. Environmental benchmarks for insecticides are based on toxicological data and an assessment factor (AF) chosen to take into account the uncertainty of extrapolating toxicity through time (acute to chronic), species, life stages and growth conditions [14]. Benchmarks, are also called EQCs depending on the regulatory framework and can be achieved following two overall methodologies. The first approach uses toxicity data of the most sensitive organism tested, such as the 50% effect concentration (EC_{50}), which is then divided by the AF [12, 15]. The second approach has become popular after the 90s and requires species sensitivity distribution (SSD) curves consisting of toxicity data for at least eight different species fitted to a cumulative distribution [16, 17]. Using the SSD approach, the concentration that affects a specific fraction of the community (usually 5%) is determined by the fitted distribution. This value is called the hazardous concentration (HCp), where p represents the affected fraction of interest [17]. The HCp is then also divided by an AF to derive the environmental benchmark concentration. The AF used for SSD is usually smaller than that of the first approach [14, 18]; it represents the sensitivity of the species community better than a randomly chosen most sensitive species. Both quality and quantity toxicity data must be evaluated for a representative number of species to construct an SSD curve [16, 17]. If there is not enough toxicity data available, the deterministic approach (AF method) is considered more appropriate [13, 19]. Apart from determining a benchmark concentration, the SSD analysis can also be used to estimate the affected fraction of species belonging to an ecosystem at a specific dichlorvos concentration, thereby assisting a quantification of the adverse effect of monitored environmental concentrations.

All four regulations cited above (US, Europe, Argentina, and China) have used AF or SSD approach to derive their regulatory concentrations for dichlorvos [9–12]. The aim of this work was, therefore: (1) to test hypotheses in terms of factors that could affect the HCp of an SSD based on literature values and to compare these with an SSD based on comparable experimental toxicity data and, (2) to compare the derived SSDs with a meta-study of environmental monitoring data. The tested hypotheses were: (i) assays using formulated dichlorvos will have lower EC_{50} values as formulated products may well include compounds with synergist effects or facilitating the uptake of dichlorvos, (ii) different taxa will have different sensitivities, being arthropods the most sensitive group, (iii) data produced under different experimental conditions will severely affect the SSD. Finally, we hypothesized that iv) environmental concentration of dichlorvos represents a risk for aquatic organisms and v) not all EQC-values are protective for the aquatic fauna.

2 Materials and methods

2.1 Chemicals

Standard dichlorvos (2,2-dichlorovinyl dimethyl phosphate) was purchased from Sigma-Aldrich (Germany), together with the reagents used to prepare the media M7 and K medium used for the maintenance of organism cultures (see Table S1 in upplemental Materials). Acetone (Baker, US, HPLC grade) was used to prepare a dichlorvos stock solution.

2.2 Cultures

The organisms used to obtain the experimental SSD were: *Daphnia magna*, *Chaoborus crystallinus*, *Chironomus riparius*, *Hyalella azteca*, *Gammarus pulex*, *Tubifex tubifex*, *Potamopyrgus antipodarum* and *Lemna minor*. They were cultured under standard conditions according to standard protocols (see Table S1). All of them were kept in the culture at the University of Copenhagen except *C. crystallinus* (which was obtained from a specialized store) and *G. pulex* that was collected in a local stream from Mølleåen, Allerød, Denmark (coordinates 55°48′58″ N 12°18′45″ E). For these, the catching method consisted of gently waving a metal 1 mm sieve submerged under native macrophytes at the river edges. Then, animals were kept in plastic containers with stream water and aquatic vegetation. Water temperature was measured and set in the climate chamber once the *Gammarus* arrived in the lab and native plants were used to feed and emulated the natural condition [20]. *G. pulex* and *C. crystallinus* were acclimated for 4 d to M7 media and standard lab light and temperature conditions [21]. During the first three days, the medium was changed gradually to increase the ratio of M7 and river water (1st day 100:0 v/v, 2nd day 50:50 v/v, and 3rd day 0:100 v/v).

2.3 Acute toxicity tests

Evaluated endpoints were immobilisation (for animals) and growth (for plants). Immobilization was defined as individuals are not being able to change their position after stimulation (manually stirring the media for 10 s). The effect is estimated as the proportion of immobilised organisms after 48 h according to the Organisation for Economic Co-operation and Development (OECD) guidelines. In the case of *L. minor*, frond growth was monitored using a digital camera. Images were taken at the beginning and the end of the incubation period (7 d). Acute tests details are given in Table 1.

Immobilisation tests were conducted in glass beakers containing 80 mL of media and a minimum of four individuals (Table 1), which were gently transferred to the beaker. Four replicate beakers were used for each dichlorvos concentration and dissolved oxygen concentration was ensured to be higher than 3 mg L^{-1} by 5 min of daily aeration. Assays of *G. pulex* and *L. minor* were carried out in six-well plates with 10 mL of media for each individual. Eighteen individuals of *G. pulex* per treatment were incubated individually in a well with a leaf (around 2 cm^2) to allow hiding behaviour. For *Lemma*, three replicates of single fronds were used.

Tests were conducted in M7 medium for animals and medium for *L. minor.* Temperature and light/darkness cycles were 20 ± 1 °C and 16:8 h, respectively for *D. magna*, *C. crystallinus*, *C. riparius*, and *P. antipodarum*. For *G. pulex* (15 ± 1 °C, 12:12 h), *T. tubifex* (20 ± 1 °C, 0:24 h) and *H. azteca* (25 ± 1 °C, 16:8 h) the conditions were adjusted to avoid temperature or light stress. The climate chamber was set at 24 ± 1 °C with a 16:8 h light-dark cycle for growing and testing of *L. minor*.

Preliminary assays have been conducted to adjust the range of dichlorvos concentration tested for the whole set of organisms. According to these results, the experiments were designed with at least five treatment concentrations, controls, and solvent controls (maximal acetone concentration < 0.01%).

2.4 Chemical analyses

Dichlorvos concentrations were evaluated by liquid chromatography coupled to a mass spectrometry detector adapted from [22]. The equipment consisted of Ultra performance liquid chromatography-tandem mass spectrometer (UPLC-MS/MS), Waters® Acquity Iclass LMS Xevo TQD and the mobile phase selected was a 70:30 volumetric mixture of formic acid 0.1% in water and formic acid 0.1% in methanol. The employed column was a Waters® ACQUITY UPLC BEHC18 (2.1 × 50 mm, particle size 1.7 µm) with a constant flow of 1.2 mL min^{-1}. The

Table 1 Experimental condition for the toxicological assays and parameters of species sensitivity distribution curves

R[a]	Species	Class[b]	Stage[c]	Time	*n* by conc.	*n*	Range (µg L^{-1})	EC$_{50}$ ± SE[c]	Slope	d (Upper value)
1	*D. magna*	Art	24 h old	48 h	4 × 5 ind[e]	200	0.022–4.60	0.22 ± 0.03	2.33 ± 0.60	0.87 ± 0.03
2	*C. crystallinus*	Art	10 mm Larvae	48 h	4 × 5 ind.	200	1.00–88	2.52 ± 0.56	1.65 ± 0.35	0.90 ± 0.03
3	*C. riparius*	Art	4th instar Larvae	48 h	5 × 5 ind.	175	2.56–40	12.0 ± 1.6	3.37 ± 1.14	0.84 ± 0.04
4	*G. pulex*	Art	RS > 5 mm	48 h	4 × 5 ind.	162	3.60–362	54 ± 14	1.62 ± 0.43	0.90 ± 0.04
5	*H. azteca*	Art	RS > 1 mm	48 h	6 × 3 ind.	140	25–460	70 ± 14	2.71 ± 1.04	0.89 ± 0.05
6	*T. tubifex*	Ann	RS > 10 mm	48 h	4 × 5 ind.	140	20–600	181 ± 44	1.39 ± 0.33	0.97 ± 0.02
7	*P. atipodarum*	Mol	RS > 1 mm	48 h	4 × 4 ind.	96	1.00–85	7320 ± 930	4.99 ± 1.83	0.95 ± 0.03
8	*L. minor*	Tra	2 weeks old	7 d	3 × 1 frond	30	1020–70,000	31,220 ± 4762	1.25 ± 0.25	0.97 ± 0.02

[a] Rank order

[b] Arthropoda (Art), Annelida (Ann), Mollusca (Mol), Tracheophyta (Tra)

[c] Random size (RS)

[d] Standard error (SE)

[e] Individuals (Ind)

retention time was 4.6 min. A six-level calibration curve was performed (see Fig. S1 in Supplementary Materials) and the relative standard deviations on samples were under 10% (Limit of quantitation (LOQ) = 10 $\mu g\,L^{-1}$).

2.5 Data collection

Literature values on acute toxicity were collected from online databases such as: ECOTOX database [23], governmental agency reports [9, 24, 25] and scientific journals by using the ScienceDirect database. The criteria used for selecting data were: i) manuscripts must be included in a specialized database; ii) the entire manuscript must be available in English and; iii) experiments must be carried out under standard procedures (including controls). From 101 references considered, 74 articles coincided with the selection criteria (Tables S2 and S3). They were divided into two groups, A and B, depending on the grade of purity of dichlorvos used. Thus, group A included 33 papers from technical grade compound (purity > 95%), the second group, B, included 41 articles that used commercial formulations of dichlorvos. In both cases, experiments were performed upon standard protocols (OECD, American Public Health Association, or similar), including controls (solvent and zero concentration), and declaring a nominal or measured concentration. From the literature database, toxicity values were also categorized into taxonomic groups (arthropods, annelids, molluscs, fishes, and anurans). Groups with low representation of species (< 8) were not considered for the taxon comparison.

Environmental concentrations of dichlorvos reported in surface waters were also reviewed. The search was done with the ScienceDirect database using the keywords: "monitoring and dichlorvos", "occurrence and dichlorvos", "surface water and dichlorvos", "water residue and dichlorvos", "pesticide and water and dichlorvos". Selection criteria were: a) no more than ten years of publication (current exposure scenarios), b) present data of recovery analysis from spiked samples c) present an analytic methodology (including control samples). Thus, 16 articles were selected where sampling sites, samples number, maximum, and mean concentration were registered for the environmental occurrence analysis.

2.6 Data analysis

Assuming normal (growth data) or binary distribution (immobility data), a three-parameter model (Eq. 1) was used to calculate EC_{50} values [26].

$$y = \frac{d}{1 + \left(\frac{c}{e}\right)^{b}} \tag{1}$$

where y corresponds to the measured variable (immobilization or relative growth), c is the dichlorvos concentration, d is the asymptotic maximum of the function (response of non-treated individuals), and e is the inflexion point of the sigmoidal function (represents the value that causes the effect in 50% of the individuals (EC_{50}). The parameter b is proportional to the slope around the EC_{50} value. Data were fitted using the open-source statistical software R version 1.1.46 and plots were performed by Sigmaplot v11.

EC_{50} values rather than No Observable Effect Concentration values were used for the SSD analysis, as they are more accurate and do not depend on the employed concentration range [27]. This criterion was used too for the bibliographic toxicological compilation data. A maximum likelihood method was applied to fit the toxicological data sets to the log-logistic model [28]. This method avoids losing censored data by reducing toxicological data to a single value instead of using their 95% confidence interval range. Using the web tool MOSAIC_SSD [28], probability distributions were fitted based on the R-package fitdistrplus. Hazardous concentrations were calculated using a bootstrap method. Finally, a hazard quotient approach was implemented to quantify how many folds the monitored values exceeded the regulatory concentrations. For that, mean and maximum concentrations were divided by the hazardous concentration for 5% of species (HC_5) obtained in the SSD analysis, and the exceedance values were discussed regarding regulatory EQC.

3 Results and discussion

3.1 Toxicity analysis

The chemical analysis on the working solutions showed all samples to stay above 80% of nominal concentrations (Table S4) and they were corrected by measured concentration. Dichlorvos was expected to be stable based on previous experiments in our group where a half-lifetime of 3 days at pH ≈ 7 was determined [22, 24].

The toxicity on aquatic organisms based on concentration-response curves is shown in Fig. 1 where all data resulted well described by a log-logistic three-parameter model (Eq. (1)). Fit parameters are given in Table 1. As is expected for an insecticide, *L. minor* was the least sensitive species having an EC_{50} value five orders of magnitude higher than the second most sensitive test species, *D. magna* (Fig. 1). The EC_{50} values were generally similar to the values presented in previous reports. For instance, Sturm and Hansen [29] reported acute EC_{50} values for *D. magna* and *C. riparius* of 0.23 and 10–20 $\mu g\,L^{-1}$, compared to the 0.22 ± 0.03

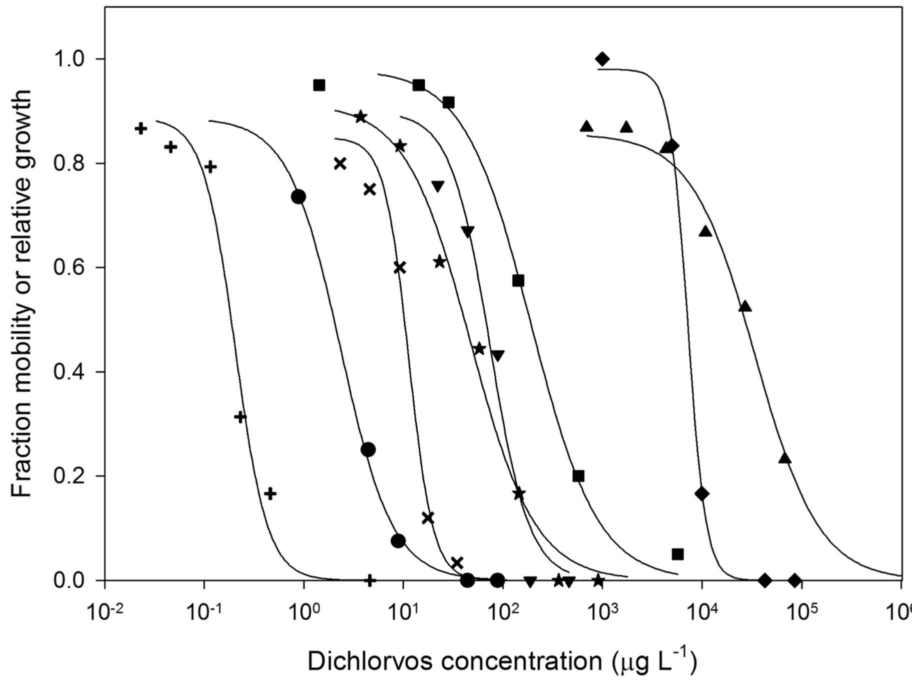

Fig. 1 Concentration-response curves for *D. magna* (+), *C. crystallinus* (●), *C. riparius* (x), *G. pulex* (★), *H. azteca* (▼), *P. atipodarum* (◆), *T. tubifex* (■) after 48 h of exposure. Concentration-response curve for *L. minor* after 7 d of exposure (▲). Control mortality was < 20% for all species and control growth rates of *L. minor* was > 0.3 d^{-1}

and 12.0 ± 1.6 μg L^{-1} reported in this study. For *Hyalella azteca*, Ankley and Collyard [30] reported an EC_{50} value of 53.3 μg L^{-1} with an exposure time of 96 h, rather than the 48 h used in our experiment giving an EC_{50} of 50 ± 14 μg L^{-1}. Johnson and Finley [31] estimated an EC_{50} of 0.5 μg L^{-1} (96 h) for *Gammarus lacustris*, which is considerably lower than the 54 ± 14 μg L^{-1} found in our study with *Gammarus pulex*. No previous reports were found for *T. tubifex*, *P. antiopdarum* and *L. minor*.

Reviewed reports on literature showed a wide range of EC_{50} values ranging from 0.07 to 57,700 μg L^{-1} (see Tables S2 and S3). Principal differences can be attributed to the studied species and the dichlorvos presentation (technical or formulation). As well, it was observed that the most studied species resulted the arthropods (crustaceans and insects). Secondly, fishes and finally, in a minority group were found molluscs, plants or algae, frogs or toads, and annelid worms.

3.2 SSD analysis

Three SSD graphs were constructed to test the hypotheses concerning causes of variability in SSDs based on: i) our experimental data, ii) literature data from assays performed with technical and formulated dichlorvos, and iii) SSD for different taxonomic groups (technical and formulated data separately for fish and arthropods) (Fig. 2). For each curve, the log-logistic model was applied and their estimated parameters are presented in Table 2. SSDs were done on acute data exposure due to the chronic studies reported not enough for this study.

All SSD curves showed good fits (R^2 > 0.95) and the quality of the fits (highest maximum likelihood values) increased with the number of species included. The experimental dataset (n = 8) showed a remarkably close fit to the SSD based on literature acute EC_{50} values for technical dichlorvos (n = 33) (Fig. 2a and b, and Table 2).

(See figure on next page.)

Fig. 2 SSD curves based on EC_{50} values are presented by groups of data. **a** Experimental SSD curve obtained in this work. **b** Comparison among technical and formulated SSD curves based on literature reports and our experimental SSD curve (this work, pink line). Taxonomic groups were arthropods (black), fishes (grey), molluscs (yellow), plants or algae (green), frogs or toads (blue), and annelid worms (red). In both cases, confidence intervals (95%) are presented in blue lines. **c** SSD curves of invertebrates and fishes for technical and formulated dichlorvos

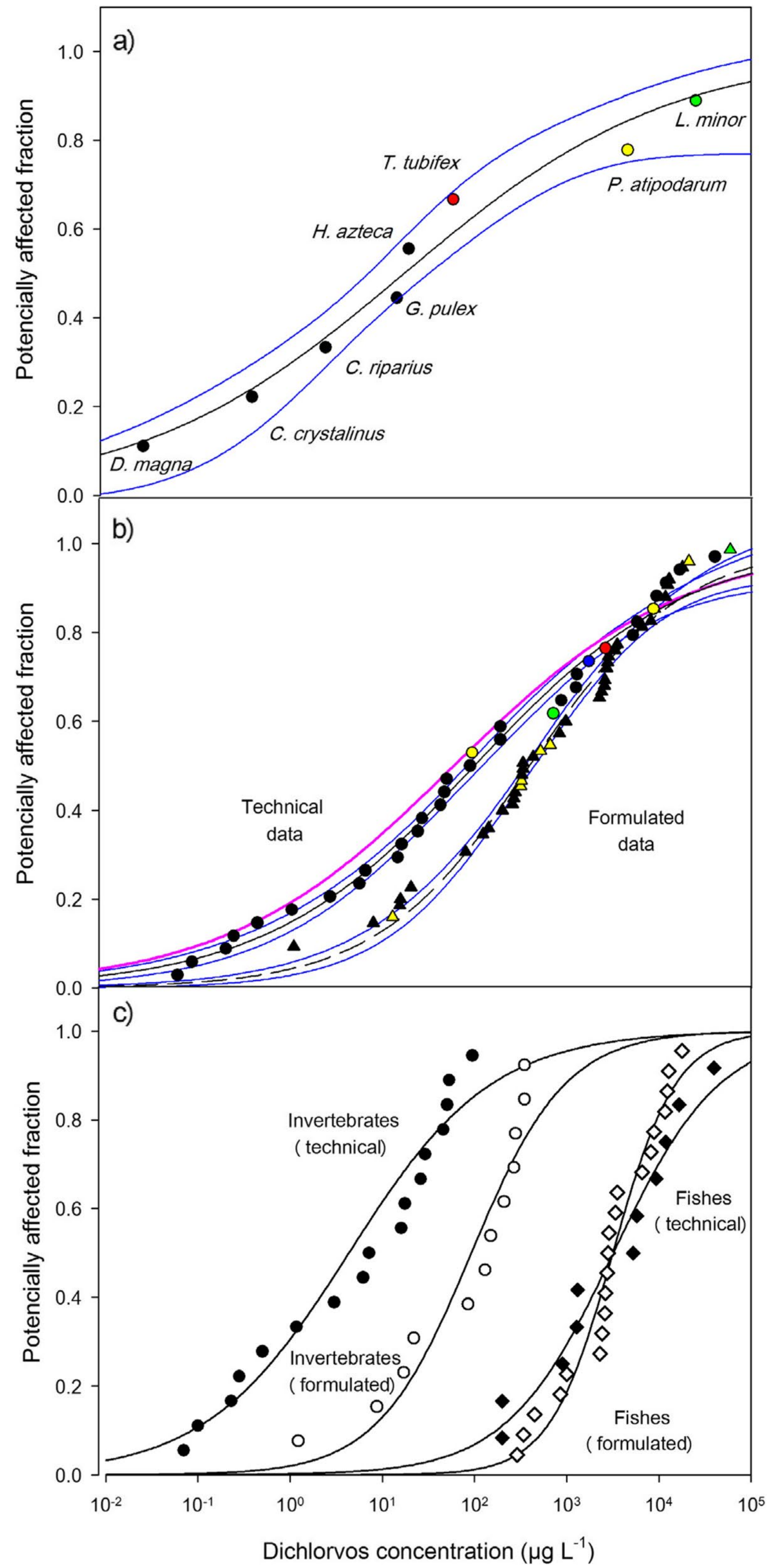

Fig. 2 (See legend on previous page.)

Table 2 Species sensitivity distribution parameters calculated by MOSAIC_SSD

Data set	Hazardous concentration		Model parameters[a]			
	HC_5 ($\mu g\,L^{-1}$)	HC_{10} ($\mu g\,L^{-1}$)	b	e ($\mu g\,L^{-1}$)	*n*	*MLV*
Experimental data (this work)	0.12 (0.0065–7.1)	0.58 (0.046–20)	0.47 (0.34–1.1)	65 (5.4–1200)	8	−30.1
Technical dichlorvos	0.084 (0.0081–0.85)	0.47 (0.066–3.30)	0.44 (0.34–0.61)	72 (18–290)	33	− 223.5
Formulated dichlorvos	13 (4–46)	38 (14–100)	0.72 (0.58–0.96)	780 (390–1600)	41	−362.4
Technical dichlorvos (Arthropods)	0.065 (0.009–0.49)	0.19 (0.035–1.00)	0.71 (0.5–1.1)	4.3 (1.3–14)	17	−60.4
Technical dichlorvos (Fish)	100 (15–680)	220 (45–1100)	0.93 (0.63–1.8)	2400 (760–1000)	11	− 104.2
Formulated dichlorvos (Arthropods)	4.8 (0.87–26)	10 (2.4–42)	1 (0.69–1.9)	87 (33–240)	12	−74.4
Formulated dichlorvos (Fish)	420 (180–1000)	690 (330–1500)	1.5 (1.1–2.3)	3100 (1800–5200)	22	−200.9

[a] Values are given with 95% confidence interval, *MLV* maximum likelihood values

As was hypothesised, there was a large and significant difference between the SSD based on technical dichlorvos and those based on the formulated (Fig. 2b and c, Table 2). This difference, however, was contrary to expected in hypothesis (i) as formulated products were less toxic compared to technical dichlorvos for the arthropods, whereas for fish, there was no difference between technical and formulated products (Fig. 2c). We have no hypotheses as to why the formulated compounds appear to be less toxic to arthropods as there was no difference for the fish species tested. The results emphasize the relevance of being critical in terms of the EC_{50} data to include in an SSD analysis. The use of quality testing criteria and broad taxonomic representation is desirable when data are available.

According to hypothesis (ii), it was confirmed that different taxa have different sensitivities being arthropods the most sensitive. Accordingly, HC-values varied 2–4 fold between arthropods and fish for formulated and technical compounds, respectively.

Moreover, a significant difference of including or excluding non-sensitive species was the slope, observing steeper slopes for fish groups and shallow slopes for datasets comprising more phylogenetic groups. The steepness of the slopes mainly affected the 95% confidence limits of the HC-values, with the smallest confidence limits being for the steepest curves. It could therefore be argued that excluding non-sensitive species could result in higher EQC-values than including them if EQC-values are based on lower confidence limits of the HC_5 as suggested by EC guideline [14]. In our case, however, EQC derived from lower confidence limits would be 6.5, 8.1, or 9.0 $ng\,L^{-1}$ for the experimental, the literature and, literature arthropods data, respectively. Using the mean HC_5 with an AF of 10 [13], the EQC would be then 12, 8.4, and 6.5 $ng\,L^{-1}$ for the same three groups, resulting in very similar values. In conclusion, as long as the data are obtained on technical compounds, making SSD on a broad range of species (including unsusceptible species) or selecting only the susceptible group makes little difference for the derived HC-values and associated dichlorvos EQC.

The correspondence between our experimentally derived SSD and that based on quality-checked literature data gives confidence in the robustness of SSD analysis. EQC-values of this study (based either the lower 95% confidence limit of the HC_5 or HC_5 divided by an AF of 10 resulted in one order of magnitude lower than the acute EQC of the USEPA [10] and Argentina [9] but corresponded to their chronic EQC of 5.8 and 7.8 $ng\,L^{-1}$, respectively. However, our calculated EQC was one and two orders of magnitude higher than the European maximum allowable concentration or annual average value [11], and one to two orders of magnitude lower than the Chinese EQC [18] (Fig. 3). Considering the relative robustness of the HC-values of our study, arthropods inclusion, and the use of technical compounds, differences in regional EQC must be due to other causes.

As long as standard protocols are applied, testing species under the same lab condition compared to bibliographic species dataset obtained could result in similar SSD curves making little difference about EQC as final result. Then the hypothesis (iii) should be discharged and causes of variation along EQC should be discussed based on data processing criterion applied to de toxicological data. For example, previous works calculating HC_5 for dichlorvos found an HC_5 of 0.0009 $\mu g\,L^{-1}$ using 27 toxicological endpoints and a total species number of 13 (mainly invertebrates species) [32]. This value is one order of magnitude lower than the

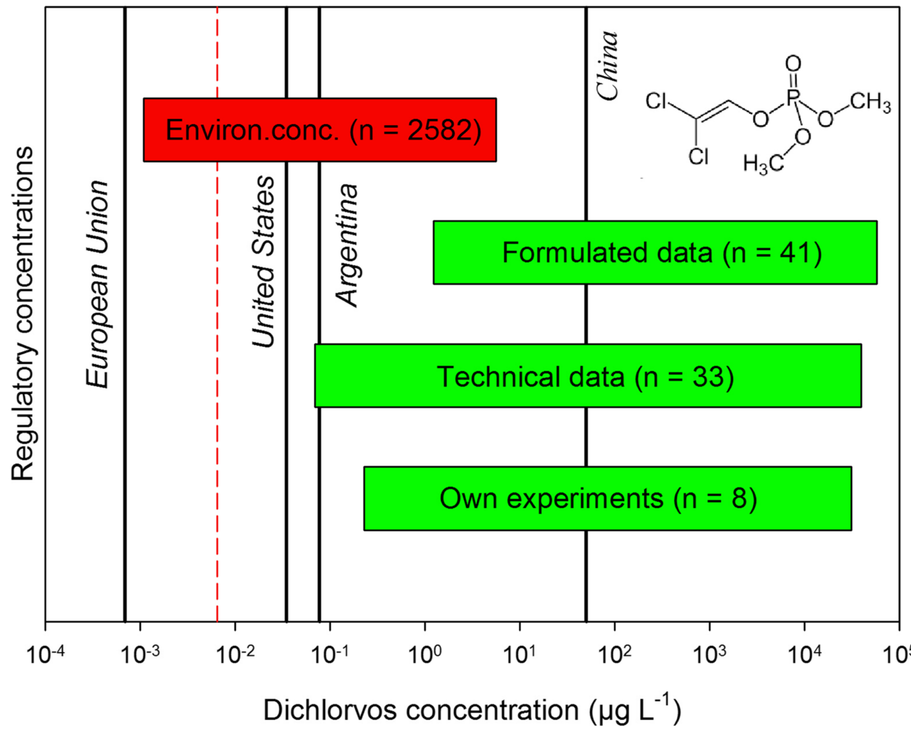

Fig. 3 Regulatory concentrations from different regions (vertical lines), proposed EQC from this study (red broke thicker line), monitored environmental concentrations range (red bar), and range of EC_{50} values (green bars)

lowest HC_5 found in our study (for technical dichlorvos on arthropods) (Table 2) and most likely stems from the inclusion of several sensible species.

He et al. [33] published two derived EQC-values according to Chinese regulations based on native and non-native species from China or the US, respectively, being 0.355 and 0.0718 $\mu g\,L^{-1}$. The USEPA and the European Commission have historically been the first agencies to set guidance values and have selected model species from their region [17, 33, 34]. Thus, geographical differences in species sensitivities cannot be ruled out. However, other comparisons between species sensitivities towards organophosphorus insecticides did not show any region-specific difference in sensitivity in mesocosm studies performed on different continents and using local species even when they were performed under distinct environmental conditions [35, 36]. The difference in the SSD of He et al. [33] is, therefore, more likely due to the selected species composition rather than on the geographical origin of the species.

One other source of variation of the EQC-values derived in different regions apart from the SSD itself is the method used to extrapolate an EQC-value from the SSD curve. As already mentioned, the estimated HC_5 and the lower confidence limit of the HC_5 are used in the European legislation with different AFs depending on the SSD-parameter applied and the level of protection desired by the several regulatory bodies. Setting regulatory benchmarks for pesticides is a political decision, taking both the cost of the pesticide in terms of risk to the environment and human health into account and its benefit to society in terms of increased agricultural outputs and eradication of vector-borne diseases. Thus, the choice of the size of the AF within different regulations might also reflect such overall risk perception [37].

3.3 Exposure concentrations and risk

Reports on environmental concentrations of dichlorvos were distributed over 10 different countries from America, Oceania, Europe, and Asia (Australia, China, Greece, India, Iran, Portugal, South Korea, Thailand, US). The occurrence of dichlorvos in environmental samples varies widely within an occurrence frequency ranging from 2 to 100%, of a total of 2582 reported samples. The affected fraction was estimated from both the mean and maximum environmental concentrations reported in Table 3. The reported concentrations of dichlorvos had presented three orders of magnitude between the lower and the highest value (0.004–5.63 $\mu g\,L^{-1}$), and the median value of the positive samples was 0.11 $\mu g\,L^{-1}$ which is lower than reported in a previous review using older references also [2]. Approximately 14% of the values listed in Table 3

Table 3 Surface water sampling data for the last 15 years. References in Supplementary materials (Table S5)

Site (#)	*n*	Freq. %	Mean $\mu g\,L^{-1}$	AF-mean	Max $\mu g\,L^{-1}$	AF-max	Sampling time
Mae Sa, Thailand	370	23	0.018	0.06	1.1	0.32	2007–2008
Haihe river, China	17	100	0.03	0.08	0.05	0.10	2008
Haraz river, Iran	8	100	1.12	0.32	1.9	0.38	May 2008
	8	100	0.64	0.27	1.4	0.35	Dec 2008
Douro river, Portugal	12	91	0.08	0.12	0.09	0.13	Mar 2009
	48		0.29	0.20	0.51	0.25	Apr 2009
	24		0.12	0.14	0.19	0.17	May 2009
Corner Inlet rivers, Australia	40	5	0.01	0.05	0.01	0.05	Summer 2009/10
Douro river, Portugal	24	100	0.06	0.11	0.87	0.3	Mar–Sep 2010
Nakdon - Han rivers, Korea	477	60	0.10	0.13	0.15	0.16	Jul–Nov 2010
Great Lakes, USA	709	8	0.041	0.09	0.29	0.20	Sep 2010–Sept 2013
Volvi lake, Greece	12	–	–	–	0.002	0.03	Oct–Nov 2010
Amravati region, India	156	–	0.186	0.17	0.25	0.19	Sep 2011–Jul 2012
Volvi lake, Greece	12	–	–	–	0.003	0.03	Mar–Jun 2011
Kosynthos river, Greece	270	4	–	–	0.027	0.08	2011–2012
Tighara, India	64	100	0.017	0.06	0.022	0.07	winter 2014
	64	100	0.004	0.03	0.012	0.05	Summer 2014
	64	100	0.005	0.04	0.010	0.05	Pre-monsoon 2014
	64	94	0.004	0.03	0.006	0.04	Post-Monson 2014
Shangyu, China	49	100	1.56	0.36	5.63	0.49	Aug 2014
Dongjiang river, China	26	100	0.004	0.03	0.014	0.06	July & Aug 2015
Shahid Rajaei dam, Iran	20	90	0.24	0.19	0.52	0.25	June 2015
	16	38	0.10	0.13	0.47	0.24	July 2015
	13	0	–	–	–	–	September 2015
	15	0	–	–	–	–	February 2016

had a concentration > 1 $\mu g\,L^{-1}$. Additionally, the findings of dichlorvos in freshwater organisms from Argentina and Belgium indicate both its occurrence in water bodies and its incorporation into biological matrices [38, 39].

Maximum concentrations were observed in China and Portugal in 2015 and 2010, respectively. Only the sampling campaign from Tighara (India) and Dongjiang River (China) presented mean concentration values below the HC_5 derived from our experimental data ($HC_5 = 6.5\,ng\,L^{-1}$), while all other mean values are higher, thereby potentially affecting more than 5% of the species and up to 36% of the species (Table 3). Not only mean and maximal values are relevant from a sampling set, also the frequency of detections due to chronic exposition represents additional risks. The meta-data in Table 3 show that at least half of the sampling data sets have a dichlorvos occurrence higher than 90%. The detection frequency is very high (even though the sampling time coincided at seasons which a high probability of occurrence) indicating close to chronic exposures for many sampling sites with little time for eco-system recovery. Besides, dichlorvos may be together by other pesticides and pollutants used in agricultural activities, making the joint environmental impact bigger than that predicted for dichlorvos alone.

4 Conclusions

We conclude that using technical dichlorvos increased toxicity compared to using formulated products, particularly in arthropods, which was unexpected. Species phylogeny also affected sensitivity and consequently the derived SSDs, but the HC_5 values used for setting regulatory concentrations were remarkably robust to the inclusion/exclusion of less sensitive species. The experimentally derived SSD was consistent with that derived from quality-checked literature values confirming that the origin of data is of less importance as long as the type and quality of the data are ensured. Assuming all regulatory bodies use data from experiments using technical dichlorvos to derive SSDs, in our opinion, regional differences in EQC values could be due to political considerations.

Our experimental SSD defined a protective concentration of 6.5 $ng\,L^{-1}$ for 5% of the species as stated by the

European values but is lower than the limit values of the US, China, and Argentina. Despite the setting of EQC's, the revision of monitoring data from the last 15 years showed extremely high occurrence frequencies of dichlorvos in concentrations higher than 6.5 $ng\,L^{-1}$ representing a risk for the environmental fauna.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42834-022-00141-y.

Additional file 1.

Acknowledgements

The authors acknowledge the support of the European Commission through Marie Skłodowska-Curie actions. Due to collaboration between the University of Copenhagen and the University of Buenos Aires, this work was developed in the Department of Plant and Environmental Sciences at the University of Copenhagen.

Authors' contributions

NC conceived the idea and supervised the entire work. NJB designed and performed experiments and collected all literature data. NC provided materials. NJB, AI, NC and AFC interpreted and discussed the data. NJB, AI and NC wrote and approved the final manuscript. The author(s) read and approved the final manuscript.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement N° 690618 KNOWPEC (Knowledge for Pesticide Control).

Availability of data and materials

All data generated or analyzed during this study are available upon request.

Declarations

Competing interests

The authors declare they have no competing interests.

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Received: 27 March 2021 Accepted: 26 May 2022
Published online: 04 June 2022

References

1. Mishra VK, Singh G, Shukla R. Impact of xenobiotics under a changing climate scenario. In: Choudhary KK, Kumar A, Singh AK, editors. Climate change and agricultural ecosystems: current challenges and adaptation. Cambridge: Woodhead Publishing; 2019. p. 133–51.
2. Sousa JCG, Ribeiro AR, Barbosa MO, Pereira MFR, Silva AMT. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J Hazard Mater. 2018;344:146–62.
3. Soare LC, Paunescu A, Maria PC. The morphophysiological, histological, and biochemical response of some nontarget organisms to the stress induced by the pesticides in the environment. In: Larramendy ML, Soloneski S, editors. Pesticides: use and misuse and their impact in the environment. London: IntechOpen; 2019. p. 1–25.
4. Ippolito A, Kattwinkel M, Rasmussen JJ, Schafer RB, Fornaroli R, Liess M. Modeling global distribution of agricultural insecticides in surface waters. Environ Pollut. 2015;198:54–60.
5. Richardson RJ. Selected neurotoxic agents – pesticides: anticholinesterase insecticides. In: McQueen CA, editor. Comprehensive toxicology. 3rd ed. Amsterdam: Elsevier; 2018. p. 308–18.
6. Kumar S, Kaushik G, Dar MA, Nimesh S, Lopez-Chuken UJ, Villarreal-Chiu JF. Microbial degradation of organophosphate pesticides: a review. Pedosphere. 2018;28:190–208.
7. Stehle S, Bline A, Bub S, Petschick LL, Wolfram J, Schulz R. Aquatic pesticide exposure in the US as a result of non-agricultural uses. Environ Int. 2019;133:105234.
8. Meftaul IM, Venkateswarlu K, Dharmarajan R, Annamalai P, Megharaj M. Pesticides in the urban environment: a potential threat that knocks at the door. Sci Total Environ. 2020;711:134612.
9. RA. Desarrollos de niveles guia nacionales de calidad de agua ambiente correspondientes a diclorvos [Developments of National Ambient Water Quality Guide Levels for Dichlorvos]. Buenos Aires: República Argentina – Subsecretaria de Recursos Hídricos de la Nación [Argentinian Republic – Department of National Water Resources)]; 2005 [in Spanish]. https://www.argentina.gob.ar/sites/default/files/documento41.pdf
10. USEPA. Pesticide Registration Review: Draft Human Health and/or Ecological Risk Assessment for Several Pesticides for DDVP, Naled, and Trichlorfon. Washington, DC: US Environmental Protection Agency; 2020.
11. EC. Directive 2013/39/EU of the European Parliament and of the Council Amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Belgium: European Commission; 2013.
12. Ding TT, Zhang YH, Zhu Y, Du SL, Zhang J, Cao Y, et al. Deriving water quality criteria for China for the organophosphorus pesticides dichlorvos and malathion. Environ Sci Pollut R. 2019;26:34622–32.
13. Sorgog K, Kamo M. Quantifying the precision of ecological risk: conventional assessment factor method vs. species sensitivity distribution method. Ecotox Environ Safe. 2019;183:109494.
14. EC. Directorate-General for Health and Food Safety. Technical Guidance for Deriving Environmental Quality Standards. Belgium: European Commission; 2018.
15. Cowan CE, Versteeg DJ, Larson RJ, Kloeppersams PJ. Integrated approach for environmental assessment of new and existing substances. Regul Toxicol Pharm. 1995;21:3–31.
16. Belanger SE, Carr GJ. SSDs revisited: part II-practical considerations in the development and use of application factors applied to species sensitivity distributions. Environ Toxicol Chem. 2019;38:1526–41.
17. Posthuma L, Suter II GW, Traas TP, editors. Species sensitivity distributions in ecotoxicology. Boca Raton: CRC Press; 2002.
18. EFSA PPR Panel. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA J. 2013;11:3290.
19. Belanger S, Barron M, Craig P, Dyer S, Galay-Burgos M, Hamer M, et al. Future needs and recommendations in the development of species sensitivity distributions: estimating toxicity thresholds for aquatic ecological communities and assessing impacts of chemical exposures. Integr Environ Asses. 2017;13:664–74.
20. Dalhoff K, Gottardi M, Rinnan A, Rasmussen JJ, Cedergreen N. Seasonal sensitivity of *Gammarus pulex* towards the pyrethroid cypermethrin. Chemosphere. 2018;200:632–40.
21. Rasmussen JJ, Cedergreen N, Kronvang B, Andersen MBB, Norum U, Kretschmann A, Strobel BW, Hansen HCB. Suspended particles only marginally reduce pyrethroid toxicity to the freshwater invertebrate *Gammarus pulex* (L.) during pulse exposure. Ecotoxicology. 2016;25:510–20.
22. Bustos N, Cruz-Alcalde A, Iriel A, Fernández Cirelli A, Sans C. Sunlight and UVC-254 irradiation induced photodegradation of organophosphorus pesticide dichlorvos in aqueous matrices. Sci Total Environ. 2019;649:592–600.
23. USEPA. The ECOTOXicology Knowledgebase. Duluth: US Environmental Protection Agency. http://cfpub.epa.gov/ecotox. Accessed 19 Sept 2019.
24. APVMA. Dichlorvos, Toxicology Assessment: the Reconsideration of Approvals of the Active Constituent, Registrations of Products Containing Dichlorvos and Approvals of their Associated Labels. Sydney: Australian Pesticides and Veterinary Medicines Authority; 2008.

25. CERI. Hazard Assessment Report. Dimethyl 2,2-dichlrovinyl Phosphate (Synonyms: Dichlorvos, DDVP). Tokyo: Chemicals Evaluation and Research Institute; 2007.
26. Jiang XG, Hansen HCB, Strobel BW, Cedergreen N. What is the aquatic toxicity of saponin-rich plant extracts used as biopesticides? Environ Pollut. 2018;236:416–24.
27. Xu FL, Li YL, Wang Y, He W, Kong XZ, Qin N, et al. Key issues for the development and application of the species sensitivity distribution (SSD) model for ecological risk assessment. Ecol Indic. 2015;54:227–37.
28. King GKK, Veber P, Charles S, Delignette-Muller ML. MOSAIC_SSD: a new web-tool for species sensitivity distribution, allowing to include censored data by maximum likelihood. Environ Toxicol Chem. 2014;33:2133–9.
29. Sturm A, Hansen PD. Altered cholinesterase and monooxygenase levels in *Daphnia magna* and *Chironomus riparius* exposed to environmental pollutants. Ecotox Environ Safe. 1999;42:9–15.
30. Ankley GT, Collyard SA. Influence of piperonyl butoxide on the toxicity of organophosphate insecticides to three species of freshwater benthic invertebrates. Comp Biochem Phys C. 1995;110:149–55.
31. Johnson WW, Finley MT. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. Washington, DC: US Fish and Wildlife Service; 1980.
32. Chevre N, Maillard E, Loepfe C, Becker-van Slooten K. Determination of water quality standards for chemical mixtures: extension of a methodology developed for herbicides to a group of insecticides and a group of pharmaceuticals. Ecotox Environ Safe. 2008;71:740–8.
33. He J, He H, Yan Z, Gao F, Zheng X, Fan J, et al. Comparative analysis of freshwater species sensitivity distributions and ecotoxicity for priority pesticides: implications for water quality criteria. Ecotox Environ Safe. 2019;176:119–24.
34. USEPA. Framework for Ecological Risk Assessment. Washington, DC: US Environmental Protection Agency; 1992.
35. Solomon K, Giesy J, Jones P. Probabilistic risk assessment of agrochemicals in the environment. Crop Prot. 2000;19:649–55.
36. Maltby L, Blake N, Brock TCM, Van Den Brink PJ. Insecticide Species Sensitivity Distributions: importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem. 2005;24:379–88.
37. Forbes VE, Calow P. Species sensitivity distributions revisited: a critical appraisal. Hum Ecol Risk Assess. 2002;8:473–92.
38. Bashnin T, Verhaert V, De Jonge M, Vanhaecke L, Teuchies J, Bervoets L. Relationship between pesticide accumulation in transplanted zebra mussel (*Dreissena polymorpha*) and community structure of aquatic macroinvertebrates. Environ Pollut. 2019;252:591–8.
39. Brodeur JC, Sanchez M, Castro L, Rojas DE, Cristos D, Damonte MJ, et al. Accumulation of current-use pesticides, cholinesterase inhibition and reduced body condition in juvenile one-sided livebearer fish (*Jenynsia multidentata*) from the agricultural Pampa region of Argentina. Chemosphere. 2017;185:36–46.

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