Ecotoxicology (2018) 27:834–844
https://doi.org/10.1007/s10646-018-1921-9

Sensitivities of three tropical indigenous freshwater invertebrates to single and mixture exposures of diuron and carbofuran and their commercial formulations

Odete Rocha[1] · Antônio José Gazonato Neto[1] · Júlio César dos Santos Lima[2] · Emanuela Cristina Freitas[2] · Mariana Miguel[2] · Adrislaine da Silva Mansano[1] · Raquel Aparecida Moreira[1] · Michiel Adriaan Daam[2,3]

Accepted: 14 February 2018 / Published online: 20 April 2018

Abstract

As compared to their temperate counterparts, few toxicity tests have been conducted so far into the evaluation of the sensitivity of indigenous tropical species to pesticides. Especially mixture toxicity assessments appear to be scarce. To contribute to increase our knowledge in this arena, we evaluated the acute toxicity of diuron and carbofuran and their mixtures to the neotropical oligochaetes *Allonais inaequalis* and *Dero furcatus*, and the ostracod *Strandesia trispinosa*. Tests were performed with both the pure active ingredients, as well as their formulated products. The toxicity of the latter to the three test organisms was generally greater than that of the pure active ingredients, although absolute differences were rather small. The sensitivity of the indigenous species was slightly greater than temperate test species from the same taxonomic groups. The concentration addition conceptual model best described the results of the mixture toxicity data. Derived deviations of this model appeared to be dependent on the test organism and as to whether the pesticides were applied as active ingredients or their commercial products. Reported field concentrations of the two pesticides indicate risks to freshwater biota, especially if they are both present. The test species used in the present study are concluded to be suitable candidates as surrogate test organisms in local pesticide risk evaluations.

Keywords Aquatic ecotoxicology · Acute toxicity · Pesticide mixtures · Tropics · Oligochaete · Ostracod

Introduction

While the agricultural soil is the primary recipient for pesticides, water bodies adjacent to crop areas are usually the ultimate recipient (Pereira et al. 2009). When compared to their temperate counterparts, edge-of-field water bodies in tropical agroecosystems have often been reported to be especially prone to pesticide contamination through runoff resulting from intensive irrigation practices and tropical rainfall (Daam and Van den Brink 2010; Lewis et al. 2016; Novelli et al. 2016). Furthermore, pesticides are often applied in close proximity to water bodies surrounding agricultural fields, resulting in relatively high levels of spray drift (Daam and Van den Brink 2010; Sanchez-Bayo and Hyne 2011).

Diuron and carbofuran are among the mostly intensively used pesticides for a wide variety of crops in Brazil (Mansano et al. 2016a). Diuron is a phenylurea herbicide that inhibits photosynthesis by blocking the electron transport chain at the photosystem II (PSII) in microorganisms and photosynthetic plants (Giacomazzi and Cochet 2004; Mansano et al. 2017). Due to its high persistence, diuron may persist in the sediment and water of contaminated aquatic environments (Field et al. 2003). Carbofuran, in

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10646-018-1921-9) contains supplementary material, which is available to authorized users.

✉ Odete Rocha
doro@ufscar.br

[1] Department of Ecology and Evolutionary Biology, Federal University of São Carlos, Rodovia Washington Luis, km 235, São Carlos SP 13565-905, Brazil

[2] NEEA/CRHEA/SHS, São Carlos Engineering School, University of São Paulo, Av. Trabalhador São Carlense, 400, São Carlos – SP 13.560-970 Brazil

[3] CENSE, Department of Environmental Sciences and Engineering, Faculty of Sciences and Technology, New University of Lisbon, Quinta da Torre, 2829-516 Caparica, Portugal

turn, is a carbamate insecticide, acaricide, and nematicide that acts against different organisms by binding to the enzyme acetylcholinesterase, inhibiting its action on acetylcholine (Moreira et al. 2015; Pessoa et al. 2011). After its application, carbofuran may be transported to a great extent from the soil to adjacent water bodies after rain events due to its high solubility and low water organic carbon sorption coefficient (Koc) levels (Ribeiro et al. 2013). Given their pesticide properties and intensive usage, several studies have indeed reported the joint occurrence of diuron and carbofuran in aquatic ecosystems in Brazil (e.g., Carbo et al. 2008; Souza 2006) and elsewhere (Bacigalupo and Meroni 2007; Faggiano et al. 2010; Masiá et al. 2015).

Regulation of the use of pesticides and characterization of their risks are usually based on studies focusing mainly on the sensitivity of a range of species representative of different trophic levels to a single chemical. In natural environments, however, organisms are constantly exposed to complex mixtures of pesticides with varying constituents in different concentrations and ratios, which may lead to additive effects or produce synergistic or antagonistic effects (Cedergreen et al. 2008; Faust et al. 2003; Mansano et al. 2017). In addition, most available studies into mixture toxicity often only evaluated the toxicity of active ingredients of pesticides, whereas commercial pesticide formulations also include surfactants and other so-called inert ingredients, which may also contribute significantly to the overall toxicity (Cedergreen and Streibig 2005; Moreira et al. 2017; Solomon and Thompson 2003).

Most available toxicity studies evaluating diuron and carbofuran have been conducted with single exposures using test species belonging to temperate regions (Mansano et al. 2016b). The relatively low availability of toxicity data for tropical species has indeed often been discussed (e.g., Daam and Van den Brink 2010; Moreira et al. 2017; Rico et al. 2011). Especially studies evaluating pesticide mixtures with tropical species are still scarce (Moreira et al. 2017; Sanches et al. 2017). A previous study conducted in our laboratory already evaluated the toxicity of both compounds individually to the neotropical cladoceran *Ceriodaphnia silvestrii* (Mansano et al. 2016b). A comparison with available literature toxicity data indicated that this species was generally the most sensitive species to both compounds and hence more sensitive than the standard test species generally used in temperate regions such as *Daphnia magna* (Mansano et al. 2016b). The toxicity of these compounds to the microalgae *Raphidocelis subcapitata* was subsequently also evaluated both individually and as a mixture in our laboratory (Mansano et al. 2017). In general, this study indicated the occurrence of synergism in the mixtures of these compounds, especially when diuron was the dominant chemical in the combinations (Mansano et al. 2017).

Aiming to contribute to increase our knowledge in pesticide mixture toxicity to tropical species, the acute toxicity of diuron and carbofuran and their commercial formulations (Diuron Nortox® 500 SC and Furadan® 350 SC), in both single and mixture exposures, was evaluated for the indigenous oligochaete species *Allonais inaequalis* and *Dero furcatus*, and for the ostracod *Strandesia trispinosa*.

Freshwater oligochaetes live on sediments and feed on decaying organic matter in most aquatic habitats, playing a substantial role in decomposition (Benbow 2009). Ostracods, although mainly epibenthic, also thrive in the water column and periphytic habitats feeding on both living and detrital suspended particles, including alga or, in the case of carnivory ostracods, other invertebrates (Rossetti et al. 2006). The use of oligochaetes as test species in ecotoxicity testing has frequently been recommended since (i) they are keystone species in aquatic ecosystems; (ii) they can be easily cultured in laboratory conditions; and (iii) they can be exposed to toxic substances through food and body contact with sediment, interstitial water, and water columns (Chapman 2001; EFSA 2013; Smith et al. 1991, Corbi et al 2015). Similarly, ostracods have also been considered functionally important components of aquatic ecosystems. They feed on many, and may demonstrate a similar or higher sensitivity to pesticides than aquatic species from other taxonomic groups (e.g., Lawrence et al. 2002; Ruiz et al. 2013; Pereira et al. 2017). Despite this, relatively few toxicity data are currently available for oligochaetes and ostracods (Daam et al. 2013; Lobo et al. 2016; Ruiz et al. 2013). The species selected for the present investigation Subsequently, the specific aims of the present studies were (i) to increase the tropical toxicity dataset with acute toxicity threshold values of diuron and carbofuran for two tropical oligochaetes and an ostracod; (ii) to compare these with toxicity data available for temperate species; (iii) to study the toxicity of mixtures of these two compounds to the species tested, and (iv) to evaluate whether single and mixture toxicity is different between the active ingredients and formulated products of these pesticides.

Materials and methods

Test organism and culture conditions

Individuals of *A. inaequalis* Stephenson, 1911 (Oligochaeta: Naididae) were obtained from laboratory cultures at the Hydraulics and Sanitation Department of the University of São Paulo and cultivated in the Ecotoxicology Laboratory Department of Ecology and Evolutionary Biology of the Federal University of São Carlos (UFSCar). Individuals of *Dero furcatus* (Oligochaeta: Naididae) were obtained from experimental tanks located at the

Aquaculture Station of the Hydrobiology Department of UFSCar and for *S. trispinosa* (Crustacea: Cypridídae) individuals were collected from concrete tanks at the Experimental Station on the campus of UFSCar.

Test organisms were kept in $25 \times 30\,cm^2$ new plastic trays containing 1 kg of artificial sterilized sediment acquired from a local aquarium store (oligochaetes) or on decomposing leaves of a local *Eucalyptus* tree (ostracod). Organisms were acclimatized to laboratory test conditions during 4 months for Oligochaeta and 1 week for Ostracoda according to the OECD protocol for Bioaccumulation in Sediment-dwelling Benthic Oligochaetes (OECD 2008). This protocol was also followed for the ostracod *S. trispinosa* due to the inexistence of a specific protocol for this species. The oligochaetes were fed once a week with 20 mL Tetramin® fish feed solution containing 5 g Tetramin® in 2 L distilled water. Cultures were kept under controlled photoperiod (16 light:8 dark) and temperature (22 ± 2 °C) in reconstituted water. Dissolved oxygen and temperature, pH, water conductivity, and water hardness were measured as recommended by the OECD Guidelines for the Testing of Chemicals (OECD 2008) at the beginning and at the end of the tests.

Chemicals, test solutions, and chemical analysis

Carbofuran (Chemical Abstracts Service (CAS), number 1563-66-2) and diuron (CAS number 330-54-1), both compounds with high purity (≥98%, analytical standard), were purchased form Sigma-Aldrich. The commercial formulations used were Furadan® 350 SC (purchased from FMC, Brazil), which contains 35% m/v active ingredient (carbofuran) and 65% m/v inert ingredients, and Diuron Nortox® 500 SC (purchased from Nortox S/A, Brazil), which is composed of 50% m/v active ingredient (diuron) and 69.4% m/v inert ingredients. The stock solutions of commercial carbofuran (100 mg active ingredient (a.i.) L^{-1}), technical carbofuran (100 mg a.i. L^{-1}), and commercial diuron (100 mg a.i. L^{-1}) were prepared immediately prior to the toxicity tests through the dilution of a predetermined amount of each compound in distilled water. The analytical standard of diuron was first dissolved in analytical-grade acetone (C_3H_6O; LabSynth) due to its low solubility in water (42 mg L^{-1} at 20 °C; Giacomazzi and Cochet 2004). The nominal test concentrations were subsequently obtained by dilution of the stock solution with the culture medium (reconstituted water) of the test organisms.

To confirm the nominal concentrations used in the tests, stock solutions were quantified using an Agilent Technologies series 1200 high-performance liquid chromatograph (Waldbronn, Germany), equipped with a diode array detector. The analytical methods were the same as those described in detail by Mansano et al. (2017).

Acute toxicity tests: isolated compounds and their mixtures

The toxicity tests followed the protocols issued by OECD (OECD 2008) and the procedures described in Corbi et al. (2015). In each acute toxicity test under static conditions (96-h exposure for *A. inaequalis* and *D. furcatus*; 48 h for *S. trispinosa* without test solution renewal), evaluating the individual active ingredients and commercial products, four replicates were used for each pesticide treatment. Each replicate consisted of a nontoxic polypropene plastic cup containing five young adult individuals (average size: 7.8 ± 0.37mm —*A. inaequalis*; 7.0 ± 0.21 mm—*D. furcatus*; 0.93 ± 0.083 mm—*S. trispinosa*) in 10 mL test solution. At least five test concentrations of each compound were evaluated, in addition to the control (and a solvent control containing 0.2% v/v acetone in the test evaluating the standard diuron solution). To certify the reproducibility of the toxicity values and take the variability of the results into account, five definitive acute toxicity tests were performed for both compounds (diuron and carbofuran) and dosing form (commercial product and active ingredient).

The test concentrations evaluated for each compound were established by conducting preliminary range-finding tests, resulting in the nominal concentration ranges provided below. Although the ranges for the active ingredients and their respective formulated products differed in some cases (as indicated by the results of the preliminary tests), the test concentrations in overlapping parts of these ranges were the same, as follows (all test concentrations evaluated in the tests are provided in Table S1 of the Supplementary Material)

Dero furcatus: 62.5 to 4000 µg L^{-1} for the active ingredient carbofuran and its formulated product Furadan® 350 SC; 2 to 64 mg L^{-1} for the active ingredient diuron and 1 to 32 mg L^{-1} for its formulated product Diuron Nortox® 500 SC.

Allonais inaequalis: 62.5 to 4000 µg L^{-1} for the active ingredient carbofuran and 500 to 32,000 µg L^{-1} for its formulated product; 2 to 64 mg L^{-1} for the active ingredient diuron and 1 to 32 mg L^{-1} for its formulated product.

Strispinosa trispinosa: 15.6 to 250 µg L^{-1} for the active ingredient carbofuran and 15.6 to 250 µg L^{-1} for its formulated product; 8 to 32 mg L^{-1} for the active ingredient diuron and 1 to 32 mg L^{-1} for its formulated product.

The experiments were conducted in a laboratory maintained at a temperature of 25 ± 1 °C, in darkness, and animals were not fed during the tests. Effects of the treatments were evaluated after 48 and 96 h for *A. inaequalis* and *D. furcatus* and only after 48 h for *S. trispinosa*. To this end, the individuals were observed under a stereomicroscope and the number of immobile organisms was counted and used to calculate the median effect concentration (96-h half-

maximal effective concentration (EC_{50}) for *A. inaequalis* and *D. furcatus* and 48 h EC_{50} for *S. trispinosa*). Water quality parameters (temperature, water hardness, electrical conductivity, and pH) were also measured at the start and at the end of each toxicity tests. In order to evaluate the combined effects of the pesticides, tests in which individuals were exposed to each pesticide compound individually and tests evaluating combinations of these compounds were conducted simultaneously through a factorial design for binary toxicity to be determined. Stock solution preparation, test conditions, and parameters analyzed were the same as those described above for the single compound exposure tests.

In addition to the single and mixture toxicity tests, the organisms were also tested monthly with the reference substance copper sulfate ($CuSO_4$). This was done in order to certify the health conditions of the tested Oligochaeta and Ostracoda and hence the validity of the toxicity tests. Reference tests were conducted in the same way as the single and mixture toxicity tests, except for the fact that no sediment was used in the reference tests.

Species sensitivity distribution

Log normal curves representing species sensitivities distribution were plotted to compare the EC_{50} values obtained from acute toxicity data of diuron and carbofuran active ingredients. Data obtained in this study for the oligochaetes *A. inaequalis* and *D. furcatus* and for the ostracod *S. trispinosa* were compared with a set of species of algae and with a selection of invertebrate species belonging to several different taxonomic groups and trophic levels. Toxicity data for algae were compiled from available literature and from the US-EPA ECOTOX database (http://cfpub.epa.gov/ecotox/) supplemented with data from the open literature and the draft assessment reports of diuron and carbofuran (EC 2004a, b) for taxa not found in the database. Only data from laboratory tests conducted at the same temperature (25°C), test duration (96 or 48 h duration), and endpoint (mortality/immobility) as our tests and that could be confirmed from original publications were included in SSDs. When more than one toxicity value attending those criteria were available for a given species, the geometric mean value was used. Log-normality at the 5% level was tested with the Anderson–Darling test included in the ETX computer program version 2.0 (Van Vlaardingen et al. 2004).

Data analysis

The 96-h EC_{50} values with their 95% confidence intervals for the acute toxicity tests were calculated by nonlinear regression through the three-parameter logistic curve in the Statistica 7.0 software (StatSoft 2004). Data were first checked for normality (χ^2 test) and homogeneity of variances (Bartlett's test). Significant differences between toxicity values derived for the active ingredients and commercial products were verified by Student's t test. The difference was considered significant when $p \leq 0.05$. All statistical analyses were performed using Sigmaplot 11.0 software (Systat 2008).

Data from the mixture toxicity tests were analyzed by comparing the observed data with the expected combined effects from concentration addition (CA) and independent action (IA) reference models using the MIXTOX tool (Jonker et al. 2005). In the following step of data analysis, the CA model was extended as recommended by Jonker et al. (2005) and deviation functions, such as synergistic/antagonistic interactions, dose-ratio-dependent and dose-level-dependent deviations were modeled by the addition of the parameters "*a*" and "*b*." The parameter "*a*" becomes negative or positive, in case of a synergism or antagonism deviation, respectively. In case of a dose-ratio-dependent deviation (DR), the value of the parameter "b_{DR}" in addition to the parameter "*a*" indicate that the deviation from the reference model is controlled by the mixture composition. The parameter "b_{DL}" is included in addition to "*a*" in the case of dose-level-dependent deviation (DL). In this deviation function, the value of "*a*" indicates the deviation at low doses (i.e., $a > 0 =$ antagonism, and $a < 0 =$ synergism) and the value of "b_{DL}" indicates at which dose level there is a change in deviation (i.e., $b_{DL} > 1 =$ at doses $<LC_{50}$ (median lethal concentration), $b_{DL} = 1$ at doses $=LC_{50}$, and $0 < b_{DL} > 1 =$ at doses $>LC_{50}$; Jonker et al. 2005).

Data were fitted to conceptual models and deviations, and the best fit was chosen by the maximum likelihood method. Where a statistically more descriptive deviation model was identified, the effect pattern was deduced directly from the parameter values and the maximum deviation was calculated in terms of effect level (Freitas et al. 2014; Jonker et al. 2005).

Results and discussion

Test performance

During all acute toxicity tests with each individual pesticide and their mixtures the measured pH of test solutions remained within the range of 7.2 and 7.8 and did not vary by more than 0.5 units in any given test. Water temperature in all toxicity tests varied between 22.8 and 24.8 °C; electrical conductivity ranged from 453.4 to 480.7 µS cm^{-1} and water hardness varied between 182 and 197 mg $CaCO_3$ L^{-1}. Survival rates >90% were observed in the control treatments at the end of all toxicity tests. In the single and mixture experiments with the active ingredient diuron, no significant

difference between the control and solvent control was noted, excluding the possibility of solvent effects (0.01% acetone) on the toxicity test results. Therefore, all validation criteria established by OECD (2008) were met.

The mean effective concentrations (96-h LC_{50}) and their 95% confidence limits for the reference substance $CuSO_4$ were 0.02575 (0.0234–0.0281) mg L^{-1} for *A. inaequalis*, 0.0314 (0.025–0.0378) mg L^{-1} for *D. furcatus* and 0.7549 mg L^{-1} (0.1424–1.3674) for *S. trispinosa*. To the best of our knowledge, no toxicity data of $CuSO_4$ are available for the three test species (nor any other species belonging to the same genus). Subsequently, the $CuSO_4$ toxicity data obtained in our study may serve as first reference values for future studies testing these species.

Measurements of diuron and carbofuran concentrations in stock solutions used in the toxicity tests showed that differences among intended and nominal concentrations were lower than 10% (mean ± SD: 97 ± 7%). Therefore, all threshold and mixture toxicity calculations were made using nominal concentrations without correction for analytical recovery as suggested by ISO (2000).

Toxicity tests with individual compounds

Regarding the acute toxicity tests for the three test organisms, the mean 48-h LC_{50} and 96-h LC_{50} values obtained for diuron and carbofuran (active ingredients and commercial formulations) and their respective 95% confidence intervals are shown in Table 1. The toxicity of the commercial formulations was significantly greater than the active ingredients in all cases except for the test evaluating the toxicity of carbofuran (standard and commercial) to the ostracod *S. trispinosa*. In the latter case, the active ingredient carbofuran was more toxic than its commercial formulation ($p < 0.05$). In line with our findings, previous studies demonstrating differences in toxicity of active ingredients and their formulated products indicated that in the majority of cases the toxicity of the formulated product was greater than that of the active ingredient (e.g., Beggel et al. 2010; Mullin 2015; Pereira et al. 2009). It should also be noted that, although statistically significant, absolute differences in the toxicity values between the active ingredients and the formulated products were rather small (Table 1).

The LC_{50} values shown in this Table 1 indicate that carbofuran was one to three orders of magnitude more toxic than diuron to the invertebrates tested. Given their insecticidal and herbicidal types of action, respectively, this was indeed in line with what could be anticipated a priori (e.g., Maltby et al. 2005; Sanchez-Bayo and Hyne 2011). Diuron is a photosynthesis inhibitor, so its main activity is on autotrophic organisms (algae and macrophytes; Van den Brink et al. 2006). Although it has been recognized that diuron may cause toxic effects on heterotrophic non-target organisms through different modes of action (e.g., *Acetylcholinesterase* inhibition, endocrine disruption activity), a specific mode of action of diuron in invertebrates remains unclear (Mansano et al. 2016a, b; Nebeker and Schuytema 1998).

In line with the above, the SSDs indicate that primary producers are less sensitive than invertebrates to carbofuran (Fig. 1a) and more sensitive than invertebrates to diuron (Fig. 1b). The three species tested for diuron in the present study were also among the most insensitive invertebrates included in the species assemblage (Fig. 1b). The most important reason that diuron was evaluated individually in the present study, however, was to enable establishing its toxicity in the mixture with carbofuran. For risk assessment purposes, primary producer toxicity data will evidently need to be used in case of the evaluation of herbicides like diuron. Regarding carbofuran, especially cladocerans (*Ceriodaphnia silvestrii* and *C. dubia*) and amphipods (three *Gammarus* spp. and *Echinogammarus tibaldii*) appear to be the most sensitive organism groups (Fig. 1a). The oligochaetes evaluated in this study appeared to have medium to low sensitivity. In line with this, laboratory and

Table 1 Mean (and 95% confidence intervals) LC_{50} values from the five acute toxicity tests with the pesticides tested as obtained for the ostracod *Strandesia trispinosa*, and the oligochaetes *Allonais inaequalis* and *Dero furcatus*

Compounds tested	*S. trispinosa* 48-h LC_{50} (mg a.i. L^{-1})	*A. inaequalis* 96-h LC_{50} (mg a.i. L^{-1})	*D. furcatus* 96-h LC_{50} (mg a.i. L^{-1})
Carbofuran (pure active ingredient)	0.024 (0.021–0.028)	1.382 (1.281–1.482)	0.314 (0.279–0.349)
Carbofuran (Furadan® 350 SC)	0.041 (0.031–0.050)	1.248 (1.209–1.287)	0.253 (0.232–0.274)
Diuron (pure active ingredient)	27.902 (16.064–39.741)	20.277 (19.505–21.04)	12.180 (11.575–12.784)
Diuron (Diuron Nortox® 500 SC)	10.479 (9.174–11.785)	15.524 (14.938–16.109)	4.611 (4.421–4.800)

LC_{50} median lethal concentration, *a.i.* active ingredient

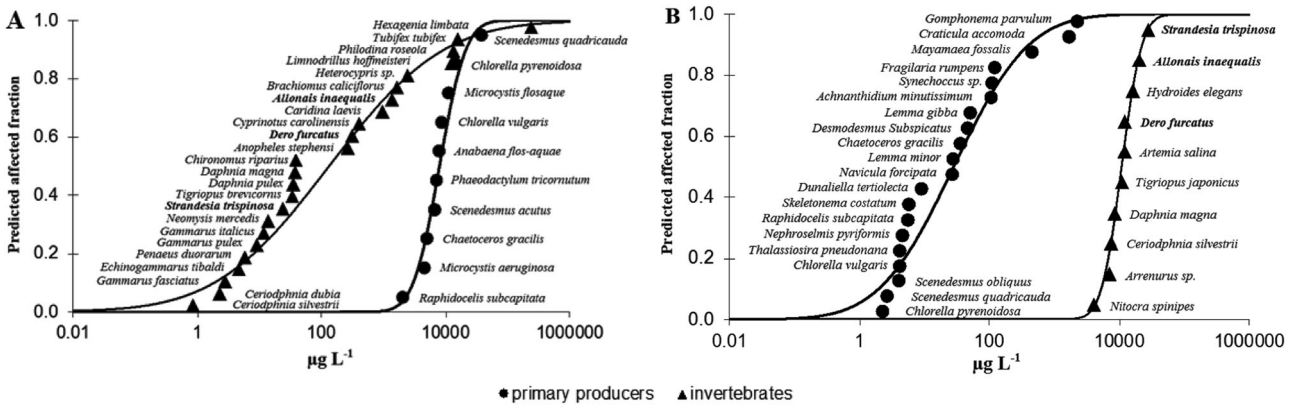

Fig. 1 Species sensitivity distributions (SSDs) constructed based on (geometric mean) 48-h EC_{50} values for carbofuran (**a**) and diuron (**b**) obtained in the present study for *Allonais inaequalis*, *Dero furcatus*, and *Strandesia trispinosa* (in bold), supplemented with data for other species from the US-EPA database (https://cfpub.epa.gov/ecotox/). SSD curves were constructed as described in Vasconcelos et al. (2016)

model ecosystem studies have indicated that cladocerans and amphipods are among the most sensitive taxonomic groups to carbamate insecticides, whereas annelids appear less sensitive (Rico and Van den Brink 2015 and references therein). On the other hand, the indigenous oligochaete species tested in the present study are more sensitive to carbofuran than the other oligochaetes for which toxicity data could be derived (*Limnodrilus hoffmeisteri* and *Tubifex tubifex*; Fig. 1a). Similarly, the indigenous ostracod (*S. tripinosa*) also appeared more sensitive than other ostracod species (*Cyprinotus carolinensis* and *Heterocypris* sp). The toxicity values obtained for *S. tripinosa* were even slightly lower than the common standard invertebrate test species *Daphnia magna* and *Chironomus riparius* (EFSA 2013; Fig. 1a). Ostracods have indeed been demonstrated to be sensitive to certain insecticides, such as organophosphates (Rico and Van den Brink 2015) and the neonicotinoid imidacloprid (Daam et al. 2013 and references therein). Although sensitivity comparisons have not demonstrated a consistent greater or lesser sensitivity of tropical species as compared to temperate species, the use of indigenous species in tropical effect assessments has been recommended for several reasons, including (i) a more ecologically relevant assessment of the true sensitivity and subsequently the potential risk of tropical freshwater life, (ii) direct availability and hence less logistic constraints, and (iii) to avoid introducing temperate exotics in tropical ecosystems (Mansano et al. 2016b and references therein).

Pesticide mixture toxicity

Traditionally, pesticide mixtures with similar modes of action are generally considered to act through CA, whereas the IA model is used for mixtures with pesticides with different modes of action (e.g., Jonker et al. 2005; Loureiro et al. 2010; Moreira et al. 2017). As detailed in the previous section, the specific mode of action of diuron in invertebrates remains unclear (Mansano et al. 2016a, b; Nebeker and Schuytema 1998). In addition, the CA model has been recommended to be used as the standard toxicity prediction tool in ecological risk assessments since the endpoints are broader in terms of organizational level, and since they focus on individual survival, reproduction output, or growth (Damasceno et al. 2017; EFSA 2014). Subsequently, the phenomenological point of view of similar mode of action states that the concept of CA could be extended to mixture constituents having common apical endpoints or common adverse outcomes (Barata et al. 2012) such as is the case in the present study (i.e., mortality). Although the CA model is generally more conservative than the IA model, the magnitude of the differences at low levels of exposure between the two models is usually small and hence, the outcome will not be overly conservative (EFSA 2013; Nørgaard and Cedergreen 2010). In analyzing the mixture toxicity results obtained from the MIXTOX tool, we concluded that the CA model better described the observed effects for the combinations tested in relation to the IA model. Although both models were statistically significant, the results obtained from the interpretation of the deviations (parameters "*a*" and "*b*") were better explained by the isobolograms produced from the CA model. Data for the results obtained for the IA model are presented as Supplementary Material in Tables S2 and S3 and in Fig. S1, whereas the results of the CA model analysis and deviations encountered for this reference model are provided in Tables 2 and 3 and visualized in Fig. 2. Based on the criteria set by Jonker et al. (2005), which are scrutinized in section "Species sensitivity distribution," the deviations of the CA model were determined for the tests with the different organisms and compound form (active ingredient and formulated product; Table 4).

Table 2 Summary of the analysis of the acute toxicity tests with the ostracod *Strandesia trispinosa* and oligochaetes *Allonais inaequalis* and *Dero furcatus* exposed to mixtures of carbofuran and diuron (active ingredients)

	S. trispinosa				*A. inaequalis*				*D. furcatus*			
	CA	S/A	DR	DL	CA	S/A	DR	DL	CA	S/A	DR	DL
Max	0.84	0.82	0.83	0.86	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98
$\beta_{carbofuran}$	2.60	2.15	2.08	1.62	3.41	3.14	3.06	2.14	3.81	3.74	3.66	2.81
β_{diuron}	2.79	6.17	5.45	4.27	4.01	4.77	5.03	3.89	2.63	2.70	2.85	1.96
LC_{50} to carbofuran	0.07	0.05	0.06	0.04	2.17	1.60	1.76	1.43	0.30	0.27	0.30	0.26
LC_{50} to diuron	40.98	35.78	34.15	33.26	37.19	29.61	27.90	27.45	16.90	15.00	13.73	15.07
a	—	1.69	2.75	3.57	—	2.17	3.61	7.03	—	0.90	2.55	4.32
$b_{DR/DL}$	—	—	−3.05	0.28	—	—	−3.20	0.34	—	—	−3.40	0.58
SS	46.89	36.23	33.98	34.32	149.01	114.53	108.31	100.24	66.13	61.63	57.16	49.57
R^2	0.83	0.87	0.88	0.88	0.83	0.87	0.88	0.89	0.92	0.93	0.93	0.94
χ^2 test or *F* test	232.17	10.66	2.25	1.91	735.91	34.49	6.22	14.28	805.57	4.50	4.47	12.06
df	—	1	1	1	—	1	1	1	—	1	1	1
p (χ^2/*F*)	4.5×10^{-49}	0.001	0.13	0.17	5.84×10^{-158}	4.29×10^{-9}	0.013	0.0002	4.8×10^{-173}	0.03	0.03	0.0005

Max maximum response value, β slope of the individual dose–response curve, LC_{50} median lethal concentration, *a*, b_{DR}, and b_{DL} parameters of the function, *SS* sum of squared residuals, r^2 the regression coefficient, χ^2 *or F* test statistic, *df* degrees of freedom, p (χ^2/*F*) significance level of the test statistic, *IA* independent action model, *CA* concentration addition, *S/A* synergism or antagonism deviation, *DR* dose-ratio-dependent deviation, *DL* dose-level deviation

Table 3 Summary of the analysis of the acute toxicity tests with the ostracod *Strandesia trispinosa* and the oligochaetes *Allonais inaequalis* and *Dero furcatus* exposed to mixtures of carbofuran (as Furadan) and diuron (as Nortox) (commercial products)

	S. trispinosa				*A. inaequalis*				*D. furcatus*			
	CA	S/A	DR	DL	CA	S/A	DR	DL	CA	S/A	DR	DL
Max	0.83	0.83	0.82	0.84	0.96	0.96	0.96	0.98	0.98	0.98	0.98	0.98
$\beta_{furadan}$	3.20	3.18	3.31	2.34	2.00	2.00	2.00	1.35	3.46	3.49	3.42	2.55
β_{nortox}	2.67	2.88	2.86	1.99	7.69	7.69	7.69	4.93	2.03	2.10	2.17	1.45
LC_{50} to furadan	0.12	0.13	0.13	0.13	5.75	5.75	5.75	3.56	0.25	0.21	0.22	0.22
LC_{50} to nortox	8.92	10.74	11.09	9.99	20.46	20.46	20.46	13.51	6.44	5.58	5.31	5.48
a	—	−1.05	−1.59	2.38	—	0.87	0.30	8.81	—	1.02	1.97	4.90
$b_{DR/DL}$	—	—	1.07	1.46	—	—	1.27	0.31	—	—	−1.82	0.55
SS	62.33	59.43	59.11	54.44	225.68	209.84	208.30	147.91	93.80	89.07	88.13	73.00
R^2	0.83	0.84	0.84	0.85	0.75	0.76	0.77	0.83	0.86	0.87	0.87	0.89
χ^2 test or *F* test	311.77	2.91	3.22	7.90	663.60	15.83	1.55	61.94	600.69	4.73	0.94	16.06
df	—	1	2	2	—	1	1	1	—	1	1	1
p (χ^2/*F*)	3.13×10^{-66}	0.09	0.20	0.02	2.66×10^{-142}	6.92×10^{-5}	0.21	3.55×10^{-15}	1.1×10^{-128}	0.03	0.33	6.1×10^{-5}

max maximum response value, β slope of the individual dose–response curve, LC_{50} median lethal concentration, *a*, b_{DR}, and b_{DL} parameters of the function, *SS* sum of squared residuals, r^2 regression coefficient, χ^2 *test or F test* test statistic, *df* degrees of freedom, p (χ^2/*F*) significance level of the test statistic, *IA* independent action model, *CA* concentration addition, *S/A* synergism or antagonism deviation, *DR* dose-ratio-dependent deviation, *DL* dose-level deviation

As can be deducted from Tables 2 through 4 and Fig. 2, different deviations from the CA model were obtained for the different test species. In the test with the ostracod *S. trispinosa* exposed to the mixture of the active ingredients, an antagonistic deviation of the CA model was encountered (Table 4). In the other mixture toxicity tests, however, a dose-level-dependent deviation could be deducted, although the dose level in relation to the LC_{50} value at which the deviation (antagonism, synergism) is expected to change differs (Table 4). Previous studies have demonstrated that PSII inhibitors such as diuron can alter the toxicity of insecticides in heterotrophs by interacting with the cytochrome P450 systems activity resulting in altered insecticide metabolism (e.g., Cedergreen 2014; Pérez et al. 2013,

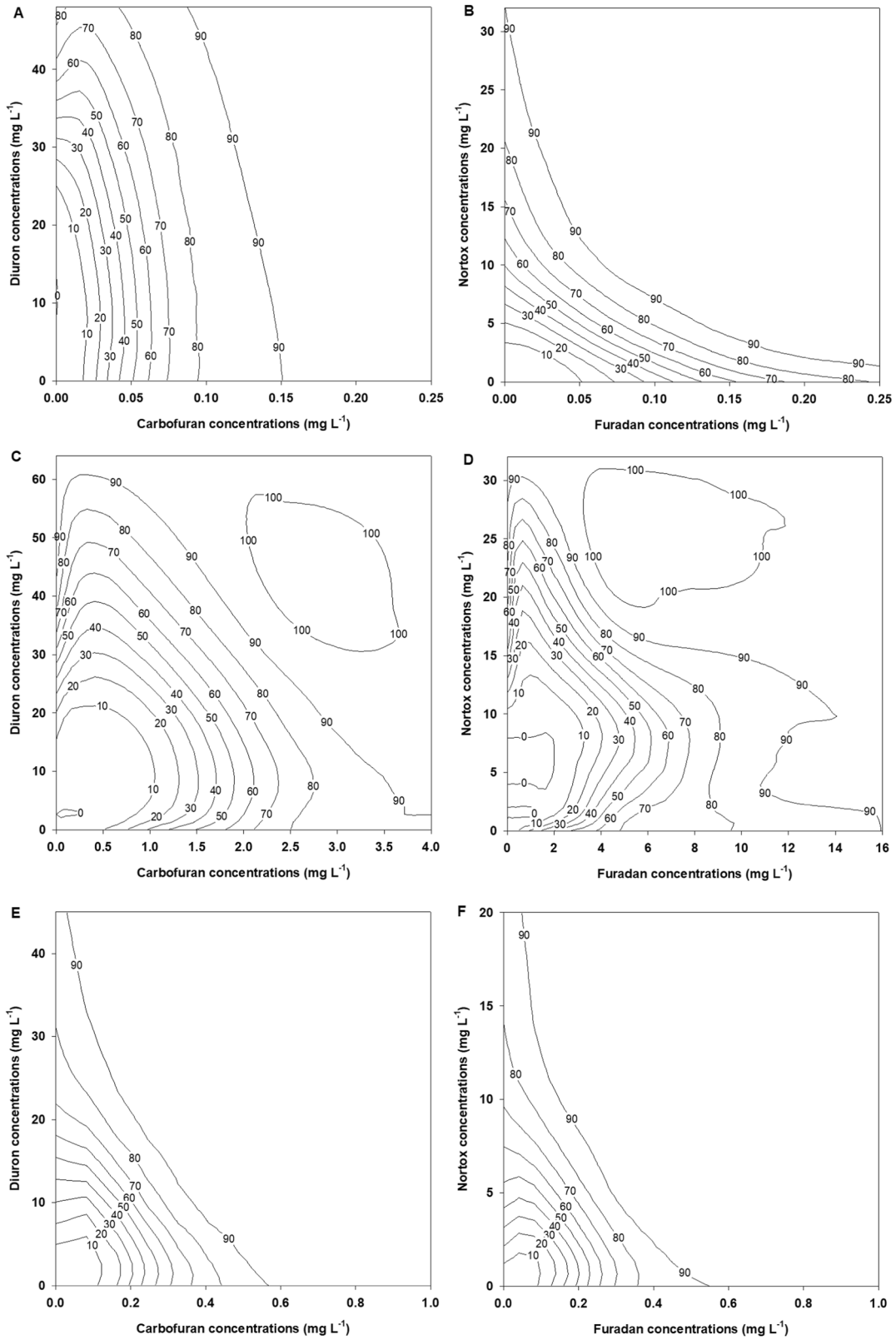

Fig. 2 Isobolograms of the effects of the mixtures of the pesticides carbofuran and diuron (**a**, **c**, **e**: active ingredients) and Furadan and Nortox (**b**, **d**, **f**: commercial formulations) on the survival of *Strandesia trispinosa* (**a**, **b**), *Allonais inaequalis* (**c**, **d**) and *Dero furcatus* (**e**, **f**). Linear, concave (numbers within the isoboles <1) and convex (numbers within the isoboles >1) isoboles in isobolograms represent no interaction, synergy, and antagonism, respectively (Ryall and Tan 2015)

Table 4 Summary of the deviations of the CA model that best fit the data of the toxicity tests with the ostracod *Strandesia trispinosa* and oligochaetes *Allonais inaequalis* and *Dero furcatus* exposed to mixtures of carbofuran and diuron and their commercial products

Species	Active ingredient	Formulated product
Strandesia trispinosa	Antagonism	DL—antagonism at low doses, synergism at higher ($<LC_{50}$) doses
Allonais inaequalis	DL—antagonism at low doses, synergism at higher ($>LC_{50}$) doses	DL—antagonism at low doses, synergism at higher ($>LC_{50}$) doses
Dero furcatus	DL—antagonism at low doses, synergism at higher ($>LC_{50}$) doses	DL—antagonism at low doses, synergism at higher ($>LC_{50}$) doses

DL dose-level deviation, LC_{50} median lethal concentration, *CA* concentration addition

2017). Since such systems differ between organisms, results obtained on the interaction of a certain binary mixture for a test organism cannot be extrapolated to other organisms, and therefore being species-specific (Liu et al. 2013). Toxicokinetic-toxicodynamic models are increasingly used in the analysis of toxicity data for single-chemical exposure (e.g., EFSA 2013). However, models of this type are also absolutely essential for a more mechanistic understanding of mixture ecotoxicology in different organisms (Jager et al. 2014). For the ostracod *S. tripinosa*, the mixture CA deviation was slightly different between the tests conducted with the active ingredients and commercial products (Table 4). At present, limited knowledge is available concerning mixture effects of pesticide formulation additives, active substances, and their transformation products (e.g., Altenburger et al. 2013; Coors et al. 2014; Moreira et al. 2017). Subsequently, future studies should also consider the influence of the "inert" ingredients in pesticide formulations on the mixture toxicity effects and mechanisms to aquatic organisms.

Implications for risk assessment and concluding remarks

Given their physical–chemical properties (e.g., high aquatic DT_{50}, low Koc), diuron and carbofuran have a great potential to be present simultaneously in edge-of-field water bodies (e.g., Field et al. 2003; Ribeiro et al. 2013). Studies carried out in Brazil (e.g., Carbo et al. 2008; Souza 2006) and in other places worldwide (Bacigalupo and Meroni 2007; Faggiano et al. 2010; Masiá et al. 2015) have indeed reported the joint occurrence of diuron and carbofuran in aquatic ecosystems. Reported carbofuran concentrations range from 0.1 to 69 $\mu g\ L^{-1}$ (Caldas et al. 2011; Carbo et al. 2008; Loro et al. 2015; Ribeiro et al. 2013), whereas maximum diuron field concentrations vary from 0.9 to 408 $\mu g\ L^{-1}$ (Britto et al. 2012; Dantas et al. 2011; Dores et al. 2009; Paschoalato et al. 2008).

Also considering the reported field concentrations of diuron and carbofuran, the indigenous species of oligochaetes and ostracod used as test organisms in this study appeared to be sensitive representatives of their taxonomic groups (Fig. 1). In addition, these organisms are functionally important components of aquatic food webs and no logistic constraints were encountered during our tests. For these reasons, oligochaetes have previously been recommended and adopted as model test species for evaluating waterborne and sediment pesticide toxicity evaluations (e.g., EFSA 2013, 2015). Subsequently, the test species used in the present study are concluded to be suitable candidates as surrogate test organisms in tropical pesticide risk evaluations.

Acknowledgements We thank the National Council for Scientific and Technological Development (CNPq) and Brazilian High Level Education Council (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)) for financial support. This work was supported by the Brazilian government through the Special Visiting Researcher program (MEC/MCTI/CAPES/CNPq/FAPs reference 402392/2013-2) and the Portuguese government (FCT) through a postdoc grant for the last author (SFRH/BPD/109199/2015) and the research unit UID/AMB/04085/2013 (CENSE).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

Altenburger R, Arrhenius A, Backhaus T, Coors A, Faust M, Zitzkat D (2013) Ecotoxicological combined effects from chemical mixtures. Part 1: relevance and adequate consideration in environmental risk assessment of plant protection products and biocides. Umweltbundesamt (UBA) Report No. UBA-FB 00, Dssau-Roßlau, Germany

Bacigalupo MA, Meroni G (2007) Quantitative determination of diuron in ground and surface water by time-resolved fluoroimmunoassay: seasonal variations of diuron, carbofuran, and paraquat in an agricultural area. J Agric Food Chem 55:3823–3828

Barata C, Fernández-San Juan M, Feo ML, Eljarrat E, Soares AMVM, Barceló D, Baird DJ (2012) Population growth rate responses of

Ceriodaphnia dubia to ternary mixtures of specific acting chemicals: Pharmacological versus ecotoxicological modes of action. Environ Sci Technol 46:9663–9672

Beggel S, Werner I, Connon RE, Geist JP (2010) Sublethal toxicity of commercial insecticide formulations and their active ingredients to larval fathead minnow (*Pimephales promelas*). Sci Total Environ 408:3169–3175

Benbow ME (2009) Annelida, oligochaeta and polychaeta. In: Reference module in earth systems and environmental sciences—encyclopedia of inland waters. Academic, New York, pp 124–127

Britto FB, Vasco AND, Pereira APS, Júnior M, Vieira A, Nogueira LC (2012) Herbicides in the upper Poxim River, Sergipe, and the risk of contamination of water resources. Rev Ciênc Agronôm 43:390–398

Caldas S, Primel E, Zanella R (2011) Risk estimate of water contamination and occurrence of pesticide in the south of Brazil. In: Kortekamp A (ed) Herbicides and environment. INTECH Open Access Publisher, Rijeka, Croatia, pp 471–492

Carbo L, Souza V, Dores EF, Ribeiro ML (2008) Determination of pesticides multiresidues in shallow groundwater in a cotton-growing region of Mato Grosso, Brazil. J Braz Chem Soc 19:1111–1117

Cedergreen N (2014) Quantifying synergy: a systematic review of mixture toxicity studies within environmental toxicology. PLoS ONE 9:e96580

Cedergreen N, Streibig JC (2005) The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard. Pest Manag Sci 61:1152–1160

Cedergreen N, Christensen AM, Kamper A, Kudsk P, Mathiassen SK, Streibig JC, Sorensen H (2008) A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. Environ Toxicol Chem 27:1621–1632

Chapman PM (2001) Utility and relevance of aquatic oligochaetes in ecological risk assessment. Hydrobiologia 463:149–169

Coors A, Weisbrod B, Schoknecht U, Sacher F, Kehrer A (2014) Predicting acute and chronic effects of wood preservative products in *Daphnia magna* and *Pseudokirchneriella subcapitata* based on the concept of concentration addition. Environ Toxicol Chem 33:382–393

Corbi JJ, Gorni GR, Correa RC (2015) An evaluation of *Allonais inaequalis* Stephenson, 1911 (Oligochaeta Naididae) as a toxicity test organism. Ecotoxicol Environ Contam 10:7–11

Daam MA, Van den Brink PJ (2010) Implications of differences between temperate and tropical freshwater ecosystems for the ecological risk assessment of pesticides. Ecotoxicology 19:24–37

Daam MA, Pereira ACS, Silva E, Caetano L, Cerejeira MJ (2013) Preliminary aquatic risk assessment of imidacloprid after application in an experimental rice plot. Ecotoxicol Environ Saf 97:78–85

Damasceno EP, Figuerêdo LP, Pimentel MF, Loureiro S, Costa-Lotufo LV (2017) Prediction of toxicity of zinc and nickel mixtures to *Artemia* sp. at various salinities: from additivity to antagonism. Ecotox Environ Saf 142:322–329

Dantas ADB, Paschoalato CF, Martinez MS, Ballejo RR, Di Bernardo L (2011) Removal of diuron and hexazinone from Guarany Aquifer groundwater. Braz J Chem Eng 28:415–424

Dores EF, Spadotto CA, Weber OL, Carbo L, Vecchiato AB, Pinto AA (2009) Environmental behavior of metolachlor and diuron in a tropical soil in the central region of Brazil. Water Air Soil Pollut 197:175–183

EC—European Commission (2004a). Draft Assessment Report (DAR)—public version—initial risk assessment provided by the Rapporteur Member State Denmark for the existing active substance diuron of the second stage of the review programme referred to in Article 8 (2) of Council Directive 91/414/EEC

EC—European Commission (2004b) Draft Assessment Report (DAR)—public version—initial risk assessment provided by the Rapporteur Member State Belgium for the existing active substance carbofuran of the second stage of the review programme referred to in Article 8 (2) of Council Directive 91/414/EEC

EFSA—The European Food Safety Authority (2013) Guidance on tiered risk assessment for plant protection products for aquatic organisms in the edge-of-field surface waters. EFSA J 11:3290.

EFSA—The European Food Safety Authority (2014) Summary report, EFSA Scientific Colloquium No. 21. Harmonization of human and ecological risk assessment of combined exposure to multiple chemicals. Edinburg, UK.

EFSA—The European Food Safety Authority (2015) Scientific opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water EFSA J 13:4176.

Faggiano L, de Zwart D, García-Berthou E, Lek S, Gevrey M (2010) Patterning ecological risk of pesticide contamination at the river basin scale. Sci Total Environ 408:2319–2326

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Grimme LH (2003) Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. Aquat Toxicol 63:43–63

Field JA, Reed RL, Sawyer TE, Griffith SM, Wigington PJ (2003) Diuron occurrence and distribution in soil and surface and ground water associated with grass seed production. J Environ Qual 32:171–179

Freitas EC, Pinheiro C, Rocha O, Loureiro S (2014) Can mixtures of cyanotoxins represent a risk to the zooplankton? The case study *of Daphnia magna* Straus exposed to hepatotoxic and neurotoxic cyanobacterial extracts. Harmful Algae 31:43–152

Giacomazzi S, Cochet N (2004) Environmental impact of diuron transformation: a review. Chemosphere 56:1021–1032

ISO—International Organization for Standardization (2000) Water quality—determination of long term toxicity of substances to *Daphnia magna* Straus (Cladocera, Crustacea). International Organization for Standardization. Geneve, Switzerland, p. 10706

Jager T, Gudmundsdóttir EM, Cedergreen N (2014) Dynamic modeling of sub-lethal mixture toxicity in the nematode *Caenorhabditis elegans*. Environ Sci Technol 48:7026–7033

Jonker MJ, Svendsen C, Bedaux JJM, Bongers M, Kammenga JE (2005) Significance testing of synergistic/antagonistic, dose level-dependent, or dose ratio-dependent effects in mixture dose–response analysis. Environ Toxicol Chem 24:2701–2713

Lawrence JR, Packroff G, Neu TR (2002) Microscale evaluation of the effects of grazing by invertebrates with contrasting feeding modes on river biofilm architecture and composition. Microb Ecol 44:199–207

Lewis SE, Silburn DM, Kookana RS, Shaw M (2016) Pesticide behavior, fate, and effects in the tropics: an overview of the current state of knowledge. J Agric Food Chem 64:3917–3924

Liu SS, Wang CL, Zhang J, Zhu XW, Li WY (2013) Combined toxicity of pesticide mixtures on green algae and photobacteria. Ecotox Environ Saf 95:98–103

Lobo H, Méndez-Fernández L, Martínez-Madrid M, Daam MA, Espíndola ELG (2016) Acute toxicity of zinc and arsenic to the warmwater aquatic oligochaete *Branchiura sowerbyi* as compared to its coldwater counterpart *Tubifex tubifex* (Annelida, Clitellata). J Soils Sediment 16:2766–2774

Loro VL, Murussi C, Menezes C, Leitemperger J, Severo E, Guerra L, Guerra L, Costa M, Perazzo GX, Zanella R (2015) Spatial and temporal biomarkers responses of *Astyanax jacuhiensis* (Cope, 1894) (Characiformes: Characidae) from the middle rio Uruguai, Brazil. Neotrop Ichthyol 13:569–578

Loureiro S, Svendsen C, Ferreira ALG, Pinheiro C, Ribeiro F, Soares AMVM (2010) Toxicity of three binary mixtures to *Daphnia magna*: comparing chemical modes of action and deviations from conceptual models. Environ Toxicol Chem 29:1716–1726

Mansano AS, Moreira RA, Pierozzi M, Oliveira TM, Vieira EM, Rocha O, Regali-Seleghim MH (2016a) Effects of diuron and carbofuran pesticides in their pure and commercial forms on *Paramecium caudatum*: the use of protozoan in ecotoxicology. Environ Pollut 213:160–172

Mansano AS, Moreira RA, Dornfeld HC, Diniz LGR, Vieira EM, Daam MA, Rocha O, Seleghim MHR (2016b) Acute and chronic toxicity of diuron and carbofuran to the neotropical cladoceran *Ceriodaphnia silvestrii*. Environ Sci Pollut Res 1–12. https://link.springer.com/article/10.1007/s11356-016-8274-9#citeas

Mansano AS, Moreira RA, Dornfeld HC, Freitas EC, Vieira EM, Sarmento H, Rocha O, Seleghim MHR (2017) Effects of diuron and carbofuran and their mixtures on the microalgae *Raphidocelis subcapitata*. Ecotoxicol Environ Saf 142:312–321

Maltby L, Blake N, Brock TCM, Van den Brink PJ (2005) Insecticide species sensitivity distributions: importance of test species selection and relevance to aquatic ecosystems. Environ Toxicol Chem 24:379–288

Masiá A, Campo J, Navarro-Ortega A, Barceló D, Picó Y (2015) Pesticide monitoring in the basin of Llobregat River (Catalonia, Spain) and comparison with historical data. Sci Total Environ 503-504:58–68

Moreira RA, Mansano AS, Rocha O (2015) The toxicity of carbofuran to the freshwater rotifer. Philos roseola Ecotoxicol 24:604–615

Moreira RA, Daam MA, Vieira BH, Sanches ALM, Reghini MV, Mansano AS, Freitas EC, Espindola ELG, Rocha O (2017) Toxicity of abamectin and difenoconazole mixtures to a Neotropical cladoceran after simulated run-off and spray drift exposure. Aquat Toxicol 185:58–66

Mullin CA (2015) Effects of inactive ingredients on bees. Curr Opin Insect Sci 10:194–200

Nebeker AV, Schuytema GS (1998) Chronic effects of the herbicide diuron on freshwater cladocerans, amphipods, midges, minnows, worms, and snails. Arch Environ Contam Toxicol 35:441–446

Nørgaard KB, Cedergreen N (2010) Pesticide cocktails can interact synergistically on aquatic crustaceans. Environ Sci Pollut Res 17:957–967

Novelli A, Vieira BH, Braun AS, Mendes LB, Daam MA, Espíndola ELG (2016) Impact of runoff water from an experimental agricultural field applied with Vertimec 18EC (abamectin) on the survival, growth and gill morphology of zebrafish juveniles. Chemosphere 144:1408–1414

OECD—Organization for Economic Cooperation Development (2008) Test no. 315: guidelines for the testing of chemicals: bioaccumulation in sediment-dwelling benthic Oligochaetes. OECD, Paris

Paschoalato C, Dantas A, Rosa I, Faleiros R, Bernardo LD (2008) Use of activated carbon to remove the herbicides diuron and hexazinone from water. Rev DAE 179:34–41

Pereira JL, Antunes SC, Castro BB, Marques CR, Goncalves AMM, Goncalves F, Pereira R (2009) Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: commercial formulation versus active ingredient. Ecotoxicology 18:455–463

Pereira AS, Cerejeira MJ, Daam MA (2017) Toxicity of environmentally realistic concentrations of chlorpyrifos and terbuthylazine in indoor microcosms. Chemosphere 182:348–355

Pérez J, Monteiro MS, Quintaneiro C, Soares AMVM, Loureiro S (2013) Characterization of cholinesterases in *Chironomus riparius* and the effects of three herbicides on chlorpyrifos toxicity. Aquat Toxicol 144:296–302

Pessoa PC, Luchmann KH, Ribeiro AB, Veras MM, Correa JRMB, Nogueira AJ, Bainy ACD, Carvalho PSM (2011) Cholinesterase inhibition and behavioral toxicity of carbofuran on *Oreochromis niloticus* early life stages. Aquat Toxicol 105:312–320

Ribeiro ACA, Dores EFGC, Amorim RSS, Lourencetti C (2013) Resíduos de pesticidas em águas superficiais de área de nascente do Rio São Lourenço-MT: Validação de método por extração em fase sólida e cromatografia líquida. Quím Nova 36:284–290

Rico A, Van den Brink PJ (2015) Evaluating aquatic invertebrate vulnerability to insecticides based on intrinsic sensitivity, biological traits, and toxic mode of action. Environ Toxicol Chem 34:1907–1917

Rico A, Waichman AV, Geber-Corrêa R, Van den Brink PJ (2011) Effects of malathion and carbendazim on Amazonian freshwater organisms: comparison of tropical and temperate species sensitivity distributions. Ecotoxicology 20:625–634

Rossetti G, Martens K, Meisch C, Tavernelli S, Pieri V (2006) Small is beautiful: diversity of freshwater ostracods (Crustacea, Arthropoda) in marginal habitats of the Province of Parma (Northern Italy). J Limnol 65:121–131

Ruiz F, Abad M, Bodergat AM, Carbonel P, Rodríguez-Lázaro J, González-Regalado ML, Toscano A, García EX, Prenda J (2013) Freshwater ostracods as environmental tracers. Int J Environ Sci Technol 10:1115–1128

Ryall KA, Tan A (2015) Systems biology approaches for advancing the discovery of effective drug combinations. J Chemin 7:1–15

Sanches ALM, Vieira BH, Reghini MV, Moreira RA, Freitas EC, Espíndola ELG, Daam MA (2017) Single and mixture toxicity of abamectin and difenoconazole to adult zebrafish (*Danio rerio*). Chemosphere 188:582–587

Sanchez-Bayo F, Hyne RV (2011) Comparison of environmental risks of pesticides between tropical and nontropical regions. Integr Environ Assess Manag 7(577):586

Smith PD, Kennedy JH, Dickson LK (1991) An evaluation of a naidid oligochaetes a toxicity test organism. Environ Toxicol Chem 10:1459–1465

Solomon KR, Thompson DG (2003) Ecological risk assessment for aquatic organisms from over-water uses of glyphosate. J Toxicol Environ Health B 6:289–324

Souza V (2006) Avaliação da contaminação de águas por resíduos de pesticidas em área de cultura de algodão: região de Primavera do Leste - MT. Doctoral thesis. Universidade Estadual Paulista, Brazil

Statsoft Inc (2004) STATISTICA, Versão 07. www.statsoft.com. Accessed 16 May 2017

Systat (2008) Systat Software, Incorporation Sigma Plot for Windows Version 11.0. Statistics for user's guide. Chicago, Systat Software Inc., 578 p

Van den Brink PJ, Blake N, Brock TCM, Maltby L (2006) Predictive value of species sensitivity distributions for effects of herbicides in freshwater ecosystems. Hum Ecol Risk Assess 12:645–674

Van Vlaardingen P, Traas TP, Wintersen AM, Aldenberg T (2004) ETX 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. RIVM report no. 601501028/2004, Bilthoven, The Netherlands

Vasconcelos AM, Daam MA, dos Santos LRA, Sanches ALM, Araújo CVM, Espíndola ELG (2016) Acute and chronic sensitivity, avoidance behavior and sensitive life stages of bullfrog tadpoles exposed to the biopesticide abamectin. Ecotoxicology 25:500–509