

Effect of Parathion-Methyl on Amazonian Fish and Freshwater Invertebrates: A Comparison of Sensitivity with Temperate Data

Andreu Rico · Rachel Geber-Corrêa · Paola S. Campos ·
Marcos V. B. Garcia · Andrea V. Waichman ·
Paul J. van den Brink

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Abstract Parathion-methyl is an organophosphorous insecticide that is widely used in agricultural production sites in the Amazon. The use of this pesticide might pose a potential risk for the biodiversity and abundance of fish and invertebrate species inhabiting aquatic ecosystems adjacent to the agricultural fields. Due to a lack of toxicity data for Amazonian species, safe environmental concentrations used to predict the ecological risks of parathion-methyl in the Amazon are based on tests performed with temperate species, although it is unknown whether the sensitivity of temperate species is representative for those of Amazonian endemic species. To address this issue, the acute toxic effect (LC₅₀-96 h) of parathion-methyl was assessed on seven fish and five freshwater invertebrate species endemic to the Amazon. These data were used to compare their pesticide sensitivity with toxicity data for temperate

species collected from the literature. The interspecies sensitivity was compared using the Species Sensitivity Distribution (SSD) concept. The results of this study suggest that Amazonian species are no more, or less, sensitive to parathion-methyl than their temperate counterparts, with LC₅₀ values ranging from 2900 to 7270 µg/L for fish and from 0.3 to 319 µg/L for freshwater arthropods. Consequently, this evaluation supports the initial use of toxicity data of temperate fish and freshwater invertebrate species for assessing the effects of parathion-methyl on Amazonian freshwater ecosystems.

Parathion-methyl [*O,O*-dimethyl *O*-(4-nitrophenyl) phosphorothioate] is a nonsystemic organophosphorous insecticide and acaricide used to control pests in a wide array of crops (ATSDR 2001). It is highly toxic to aquatic organisms and has been classified as “extremely hazardous” for the environment by the WHO (2004). Its use has been banned or restricted in 23 countries and its import is illegal in a total of 50 countries (PAN 2009). Despite the high toxicity of this compound, several studies have shown that parathion-methyl is intensively used among fruit and vegetable producers in floodplain areas of the Brazilian Amazon (Waichman et al. 2002, 2007). Amazonian farmers use higher application rates, more applications per production cycle, and shorter intervals between applications than recommended by the producing companies and the Brazilian Pesticides Law (Law 7.802/89). Waichman et al. (2007) pointed out that the lack of technical support and training of Amazonian farmers are among the causes of the indiscriminate use of this pesticide, which is frequently accompanied by incorrect practices (i.e., washing of application equipment in the river, inappropriate practices of container disposal, etc.). The incorrect use of pesticides

A. Rico (✉) · P. J. van den Brink
Department of Aquatic Ecology and Water Quality
Management, Wageningen University and Research Centre,
P.O. Box 47, 6700 AA Wageningen, The Netherlands
e-mail: Andreu.Rico@wur.nl

R. Geber-Corrêa · P. S. Campos · A. V. Waichman
Universidade Federal do Amazonas, Instituto de Ciências
Biológicas, Av. Rodrigo Otávio Jordão Ramos 3000, 69077-000
Manaus, Amazonas, Brazil

M. V. B. Garcia
Embrapa Amazônia Ocidental, Rod. AM-010, km 28, 69010-970
Manaus, Amazonas, Brazil

P. J. van den Brink
Alterra, Centre for Water and Climate, Wageningen University
and Research Centre, P.O. Box 47, 6700 AA Wageningen,
The Netherlands

is one of the main sources of pollution of aquatic ecosystems in the Amazon, posing a potential risk not only for the biodiversity and abundance of freshwater organisms but also for the sustainability and functioning of aquatic ecosystems. Consequently, there is a need to assess the ecological risks that the contamination of pesticides such as parathion-methyl represents to Amazonian freshwater ecosystems.

Due to a lack of site-specific toxicity data, in many tropical and subtropical countries like Brazil, the ecological risk assessment and the calculation of safe environmental concentrations for the control and monitoring of pesticides in the aquatic ecosystems have relied on European and North American single-species toxicity bioassays (Kwok et al. 2007; Lacher and Goldstein 1997). Consequently, acceptable environmental concentrations for pesticides (i.e., parathion-methyl) used in the Brazilian Amazon are based on the extrapolation of toxicity data derived in the temperate region; hence, differences in environmental parameters and species sensitivity are not being taken into account (Römbke et al. 2008). Differences in sensitivity between species should be assessed and incorporated in the risk assessment, because an extrapolation of temperate data to tropical conditions without a scientific base could lead to a potential risk for tropical freshwater ecosystems (Henriques et al. 1997; Lacher and Goldstein 1997). The differences in sensitivity to pesticides between freshwater species from different climatic regions have been already studied by several authors (Daam et al. 2008, 2009; Dyer et al. 1997; Kwok et al. 2007; Maltby et al. 2005). These studies could not demonstrate significant differences in sensitivity to pesticides between temperate and tropical species. Most of the tropical species used in this comparison, however, belong to the tropical region of Asia, and the uncertainty in the extrapolation of toxicity data calculated for species of the temperate region to protect Amazonian ecosystems still remains an open question. To address this issue, the present study aimed to estimate the effect of parathion-methyl on Amazonian endemic aquatic organisms (i.e., fish and freshwater macroinvertebrates) in order to study possible systematic sensitivity differences between Amazonian and temperate species. The results of this approach will be used to provide a recommendation on the applicability of temperate data in the derivation of safe environmental concentrations for parathion-methyl in the Amazon.

Material and Methods

Acute Toxicity Tests

The effect of parathion-methyl on Amazonian fish and macroinvertebrate species was assessed by performing

Table 1 Taxonomic classification, stage, length and weight (mean \pm SD; $n = 10$) of tested fish and invertebrate species

Class, order, family, and species	Stage	Length (cm)	Weight(g)
<i>Fish</i>			
Characiformes			
Characidae			
<i>Colossoma macropomum</i>	Alevin	3.1 \pm 0.3	0.3 \pm 0.1
<i>Hypphessobrycon erythrostigma</i>	(Sub) Adult	4.1 \pm 0.5	0.9 \pm 0.3
<i>Paracheirodon axelrodi</i>	(Sub) Adult	2.8 \pm 0.4	0.4 \pm 0.3
Lebiasinidae			
<i>Nannostomus unifasciatus</i>	(Sub) Adult	3.9 \pm 0.3	0.4 \pm 0.1
Perciformes			
Cichlidae			
<i>Dicrossus filamentosus</i>	(Sub) Adult	3.0 \pm 0.5	0.3 \pm 0.2
Siluriformes			
Callichthyidae			
<i>Corydoras pygmaeus</i>	(Sub) Adult	2.1 \pm 0.4	0.2 \pm 0.1
Loricariidae			
<i>Otocinclus affinis</i>	(Sub) Adult	3.1 \pm 0.5	0.5 \pm 0.2
<i>Crustacea</i>			
Decapoda			
Palaemonidae			
<i>Macrobrachium ferreirai</i>	Adult	3.8 \pm 0.6	1.2 \pm 0.4
<i>Insecta</i>			
Coleoptera			
Hydrophilidae			
<i>Hydrophilus</i> sp.	(Sub) Adult	0.8 \pm 0.1	< 0.1
Hemiptera			
Notonectidae			
<i>Buenoa unguis</i>	Adult	0.6 \pm 0.1	< 0.1
Lepidoptera			
Arctiidae			
<i>Palustris laboulbeni</i>	Larvae	3.5 \pm 0.9	0.6 \pm 0.3
<i>Gastropoda</i>			
Mesogastropoda			
Ampullariidae			
<i>Pomacea dilioides</i>	(Sub) Adult	1.6 \pm 0.3 (\emptyset)	1.5 \pm 0.3

short-term toxicity tests (LC₅₀–96 h) with the species shown in Table 1. Fish individuals were purchased from a commercial source and were acclimatized for at least 7 days to laboratory conditions. Invertebrates were collected from uncontaminated shallow lakes, ponds or streams in the vicinity of Manaus, Brazil. Criteria used to select the

studied species were based on availability through the year, ability to be kept under laboratory conditions, and body size (mainly for fish). The life-cycle stage, length, and weight of the individuals tested are given in Table 1.

All tests were performed with an emulsifiable concentrate formulation of the commercial product Folisuper 600® (a.i. parathion-methyl 600 g/L). Stock solutions were made by diluting the test compound in distilled water and test media were prepared by diluting the stock solutions in tap water collected at the experimental facilities of Embrapa Amazônia Ocidental, Brazil. The tap water contained a low concentration of nutrients (0.005 mg/L NH₄⁺ and 0.05 mg/L PO₄-P), had an alkalinity of 2.2–2.3 mg/L of CaCO₃, and had similar chemical characteristics to the stream water of fish and invertebrates habitats. Because *Pomacea dilioides* is not capable of living in extremely soft waters, the water used for these tests was collected from the uncontaminated pond from which they were collected. This water was previously filtered through a prewashed glass fiber filter (Whatman GF/C, pore size = 1.2 μm) and had a higher nutrient content (0.3 mg/L NH₄⁺ and 6.7 mg/L PO₄-P) and higher alkalinity (63.1 mg CaCO₃ /L) than the tap water used for the rest of experiments.

All single-species tests were set up as static tests with a single application of parathion-methyl. Because parathion-methyl shows a fast dissipation under field conditions (Schulz et al. 2003), the exposure regime in static tests was considered more appropriate than a constant exposure. The

tests were performed in 2.5-L glass vessels with five treatment levels and an untreated control. All tests were done in triplicate ($n = 3$) with 10 individuals per test unit. Intervals between nominal concentrations in the test range differed by a factor of 1.5–2.2. OECD guidelines for testing the acute effects of chemicals on aquatic organisms (OECD 1992) were adapted to the natural conditions of the Amazonian species; that is, the water used in the experiments was similar to their habiting waters: soft water (2–3 mg CaCO₃/L), acid pH (5.5–6.5), and oxygen concentration higher than the 60% of saturation value.

The tests were conducted in a temperature-controlled room (26 ± 1°C) with a light/dark regime of 12 h light and 12 h darkness. During the tests, the temperature of the media remained within the limits given in Table 2. In order to ensure a sufficient oxygen concentration in the test medium, an aeration system was installed in the experiments performed with fish and *Macrobrachium ferreirai*. The rest of invertebrate species are air-breathers or are capable of combining both respiration types so the tests were performed without aeration of the water. Dissolved oxygen concentrations and pH were measured with an YSI MODEL 550A meter and an YSI 100 pH meter, respectively, at 2 h, 24 h, 48 h, 72 h, and 96 h of exposure in at least one test unit per treatment level (including controls). Water hardness was measured before and after pesticide application and at the end of the experiment in some of the tests units, chosen randomly. Results of these measurements are reported in Table 2.

Table 2 Results of the acute toxicity tests with the insecticide parathion-methyl, volume of test units, and water parameters

Species	LC ₅₀ -96 h (μg/L) (95% confidence limits)	Test unit volume (L)	Temperature (°C) mean ± SD	pH, min-max	O ₂ (mg/L), min-max
Fish					
<i>Colossoma macropomum</i>	4983 (4410–5631)	2.5	26.4 ± 0.6	6.6–8.1	4.2–6.4
<i>Hyphessobrycon erythrostigma</i>	7270 (7053–7493)	2.5	26.8 ± 0.5	6.6–8.0	4.0–6.5
<i>Paracheirodon axelrodi</i>	6091 (5487–6762)	2.5	26.4 ± 0.2	5.3–7.2	4.8–6.9
<i>Nannostomus unifasciatus</i>	5385 (5018–5779)	2.5	26.7 ± 0.2	6.1–7.8	4.1–6.7
<i>Dicrossus filamentosus</i>	2900 (2656–3166)	2.5	25.9 ± 0.2	5.3–6.4	4.0–6.0
<i>Corydoras pygmaeus</i>	4093 (3823–4382) ^a	2.5	26.0 ± 0.2	5.5–6.6	4.0–6.1
<i>Otocinclus affinis</i>	6829 (6110–7632)	2.5	27.3 ± 0.3	7.1–8.5	4.0–6.4
Invertebrates					
<i>Macrobrachium ferreirai</i>	73.1 (62.5–85.5) ^a	2.0	27.7 ± 0.4	6.3–8.1	4.2–6.2
<i>Hydrophilus</i> sp.	41.4 (29.2–58.9)	1.0	26.9 ± 0.5	6.6–7.4	–
<i>Buenoa unguis</i>	0.30 ^{b,c}	1.0	26.4 ± 0.4	5.6–7.1	–
<i>Palustra laboulbeni</i>	319 (263–387)	1.0	26.6 ± 0.5	6.2–7.5	–
<i>Pomacea dilioides</i>	13607 (12164–15221)	1.0	26.5 ± 0.3	6.7–8.3	–

^a Indicative because of cannibalism in controls (cannibalism is not indicated as a negative response)

^b LC₅₀ calculated at 72 h of exposure due to high mortality in controls

^c Confidence limits not available due to singularity in regression model

Mortality was chosen as an end point for assessing the effect of parathion-methyl on fish and macroinvertebrates. Lethal effects were monitored 2 h, 24 h, 48 h, 72 h, and 96 h after exposure and dead organisms were removed from the experiment. For fish and macroinvertebrates, individuals were scored as dead when no response of any kind was observed for about 30 s after repeated tactile stimulation with a pair of metal forceps. Tests were rejected when mortality in the controls exceeded 10% for fish and 20% for invertebrates.

Calculation of LC₅₀ for Amazonian Species

The LC₅₀–96 h values and their confidence intervals were calculated by a log concentration – probit effect regression model with the ToxRat Professional Version 2.07 program (ToxRat 2003), using the nominal concentrations calculated for the experiments. Abbott's formula was used by the program to correct for mortality in the untreated controls. Confidence intervals could only be calculated when at least one partial response between 0% and 100% effects was observed (singularity in regression model).

Selection of Toxicity Data for Temperate Species

The toxicity data of parathion-methyl for temperate species (27 fish and 17 freshwater arthropods) used in this study was the same used by Maltby et al. (2005), which had been collected from existing toxicity databases (e.g., www.epa.gov/ecotox and De Zwart 2002). In their study, single-species toxicity (EC₅₀ and LC₅₀) data for fish and freshwater invertebrates with exposure duration between 2 and 21 days for fish and 1 and 7 days for invertebrates were selected. When more than one toxicity value was reported for the same species or various values were provided for a genus with no specific species name, the geometric mean was calculated.

Comparison of Pesticide Sensitivity

The sensitivity of freshwater Amazonian and temperate species to parathion-methyl was compared through the Species Sensitivity Distribution concept (SSD). The SSD can be defined as a cumulative distribution function of the toxicity of a compound to a set of species that have a common characteristic such as taxonomic group, habitat, or geographical region (Posthuma et al. 2002). The SSD model has been traditionally used in prospective risk assessment to calculate hazardous concentrations (HCs)—concentration of chemical in the water that will affect a proportion (p) of species (HC p). The cutoff value of the 5%

of species of the left tail of the distribution (HC₅) has been traditionally used to derive safe environmental concentrations, assuming that ecosystems can tolerate a certain degree of chemical stress (Posthuma et al. 2002). In the present study, SSD analyses for fish and freshwater invertebrates were conducted according to the method of Aldenberg and Jaworska (2000) and performed using the ETX 2.0 software (Van Vlaardingen et al. 2004). The spreadsheet calculates the HCs for the 5% and 50% of species (HC₅ and HC₅₀, respectively) and their 95% confidence limits. The model assumes a log-normal distribution of the introduced L(E)C₅₀ toxicity data according to the formula

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} * \exp\left(\frac{-0.5 * (x - \mu)^2}{\sigma^2}\right),$$

where $\chi = \log(\text{EC}_{50} \text{ or } \text{LC}_{50})$, μ is the median of $\log(\text{EC}_{50} \text{ or } \text{LC}_{50})$, and σ is the standard deviation of $\log(\text{EC}_{50} \text{ or } \text{LC}_{50})$.

The SSD was defined as the cumulative frequency distribution of toxicity data as follows:

$$F(x) = \int_{-\infty}^x f(x) ds.$$

Tests for log-normality were performed by means of the Anderson–Darling goodness-of-fit test. Normality of toxicity data was assumed at $p \geq 0.05$ (Posthuma et al. 2002).

The temperate invertebrates dataset was composed only by freshwater arthropods because large differences in pesticide sensitivity between arthropods and nonarthropods invertebrates are expected (Maltby et al. 2005). Therefore, only toxicity data for arthropods were included in the sensitivity distribution of Amazonian invertebrates, which are still the majority of the tested species because only one species is not an arthropod (*Pomacea dilioides*).

The sensitivity of Amazonian and temperate fish and freshwater arthropods was compared with the SSD concept. Sensitivity distributions were compared by using the two-sample Kolmogorov–Smirnov test, a nonparametric test that is used to assess whether two samples come from the same distribution, calculated with the SYSTAT 12 statistical package (SYSTAT 2007).

Results and Discussion

Acute Toxicity Tests

The results of acute toxicity tests performed in this study and test water conditions are shown in Table 2. The lower pH values corresponded with the measurements made right

after application. Higher pH values could have been induced by the release of small amounts of ammonia present in the pesticide formulation and the rapid decomposition of dead organisms into the test units. The oxygen concentration of the test media seemed to depend on the size and metabolism of the organisms and the efficacy of the aeration system. Water hardness ranged from 2 to 3 mg CaCO₃/L. A possible relationship between concentrations of parathion-methyl and the pH, hardness, and dissolved oxygen concentration was not observed. For *Buenoa unguis*, mortality in controls became higher than 20% after the third day of exposure, forcing us to calculate the lethal concentration for 72 h. Cannibalism was observed for *Corydoras pygmaeus* (fins bitten) and *Macrobrachium ferreirai* (damage produced by pincers) in controls; however, it was not counted as a negative effect because it occurred homogeneously in all treatments and mortality at controls did not exceed 10% and 20%, respectively.

Of all species tested, *B. unguis* (Hemiptera) was the most sensitive, followed by *Hydrophilus* sp. (Coleoptera), *M. ferreirai* (Crustacea), and *P. laboulbeni* (Lepidoptera). Amazonian arthropods (insects and crustaceans) were found to be highly sensitive to parathion-methyl with acute LC₅₀s ranging from 0.3 to 320 µg/L. These results are similar to other studies performed with temperate species exposed to this compound and different organophosphorous insecticides, probably due to the same mode of action of those pesticides (Maltby et al. 2005; Van Wijngaarden et al. 2005). Within fish, *Dicrossus filamentosus* was found to be the most sensitive species, followed by *Corydoras pygmaeus* and the alevins of *Colossoma macropomum*. LC₅₀ values ranged from 2.9 to 7.3 mg/L for Amazonian fish species. *Pomacea dilioides* (Gastropod) was found to be the most resistant species to parathion-methyl. They responded to the exposure to parathion-methyl by closing their operculum as an avoidance behavior, reducing their

metabolic rate, exchange with the media, and, hence, their exposure. Several authors have already pointed out that this avoidance behavior is one of the main causes that influence the high tolerance of mollusks to pesticides (Daam et al. 2009; Schroer et al. 2004).

The internal validity of the results was acceptable because the confidence intervals of the toxicity values are relative small (lower and upper confidence limits differed by no more than a factor of 2), with the exception of *B. unguis*, whose confidence intervals could not be calculated due to the singularity of the regression model. On the other hand, the external validity of these results becomes difficult to assess because there are no previous ecotoxicological studies available with parathion-methyl for the tested species.

Comparison of Pesticide Sensitivity Between Amazonian Species and Temperate Species

The SSD curves for Amazonian and temperate freshwater species are shown in Fig. 1. The sensitivity distributions of temperate and Amazonian species did not show significant differences for fish (two-sample Kolmogorov–Smirnov test: $k = 0.30$, $n_1 = 27$, $n_2 = 7$, $p = 0.68$) or for arthropods ($k = 0.63$, $n_1 = 17$, $n_2 = 4$, $p = 0.10$). The Anderson–Darling goodness-of-fit test was accepted for all four samples of toxicity data.

Table 3 shows the median values of HC₅ and HC₅₀ for temperate and Amazonian fish and arthropods with their lower (95%) and upper (5%) confidence limits. Median hazardous concentrations for temperate species appeared to be slightly lower than the values calculated for Amazonian species, with the exception of the median HC₅ value for temperate arthropods; however, confidence intervals overlapped in all four cases.

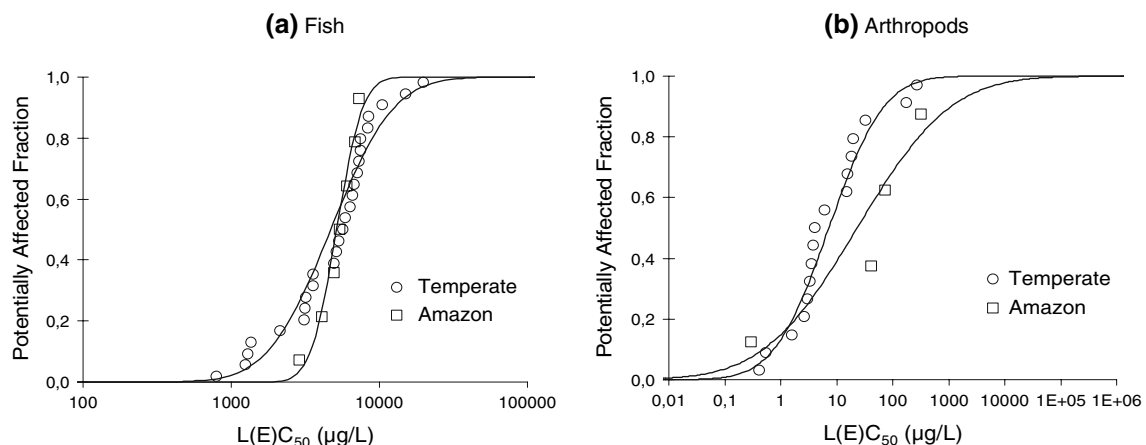


Fig. 1 SSDs for temperate (circles) and Amazonian (squares) species of **a** fish and **b** freshwater arthropods exposed to parathion-methyl

Table 3 Median hazardous concentrations of parathion-methyl for 5% and 50% of species (HC₅ and HC₅₀, respectively; in µg/L) and their lower (95%) and upper (5%) confidence limits for each SSD showed in Fig. 1

	Temperate	Amazon
Fish		
HC ₅	1308 (827–1824)	2963 (1739–3839)
HC ₅₀	4697 (3650–6044)	5152 (4074–6514)
Arthropods		
HC ₅	0.37 (0.09–0.96)	0.09 (0.00004–2.45)
HC ₅₀	7.26 (3.43–15.4)	23.2 (0.66–814)

The results of the present study are in accordance with the study made by Dyer et al. (1997), who did not find significant differences between toxicity data for tropical and temperate fish exposed to four pesticides (carbaryl, DDT, lindane, and malathion) and two phenolic compounds (phenol and pentachlorophenol), and with the results of Maltby et al. (2005), who could not demonstrate significant differences between the SSDs constructed with aquatic arthropods from the tropical region and the temperate region for three insecticides (chlorpyrifos, fenitrothion, and carbofuran). These results seem to contradict the hypothesis that tropical organisms are more susceptible to pesticide exposure than their temperate counterparts due to an increase of temperature and metabolic rates (Castillo et al. 1997; Peters et al. 1997). This hypothesis has been supported by temperature–effect studies developed for a single species and might not explain adequately the variation of pesticide sensitivity between species from different climatic regions.

Kwok et al. (2007) conducted the most extensive comparison between temperate and tropical species sensitivity to chemical substances. In this study, SSDs were constructed for 18 compounds, including nutrients, metals, narcotics, and pesticides. For the studied pesticides, inherent sensitivity differences between temperate and tropical freshwater organisms could not be demonstrated; however, a safety factor of 10 was recommended when surrogate temperate Water Quality Criteria are used in tropical and subtropical regions. In the present study, according to the comparison of threshold concentrations (HC₅) derived from the SSDs, safe environmental concentrations for parathion-methyl constructed with temperate data resulted on a sufficient protection level to Amazonian species and, hence, the extrapolation factor proposed by Kwok et al. (2007) would not be necessary. However, according to the studies of Newman et al. (2000) and Wheeler et al. (2002), the number of Amazonian toxicity data included in the present study is rather small to ensure a minimal uncertainty in the SSD approach. Furthermore, considering the large diversity of fish and

invertebrate species present in the Amazonian waters, species with different traits and adapted to specific environmental conditions (i.e., hypoxic or even anoxic water conditions) that could show different responses to exposure to parathion-methyl might be misrepresented in the Amazonian dataset used in the present study. Consequently, a wider array of toxicity studies for different Amazonian freshwater species should be performed in order to increase the confidence of the interspecies sensitivity comparison for parathion-methyl. On the other hand, several studies conducted in the temperate region have tested the potential of HC₅ values derived from SSDs calculated with single-species laboratory toxicity tests in protecting freshwater communities (Maltby et al. 2005; Schroer et al. 2004; Versteeg et al. 1999). These studies demonstrated that the HC₅ values (5% of affected species) are protective of adverse effects in freshwater model ecosystems for the application of pesticides and are a useful tool for deriving safe environmental concentrations. However, this assumption has not been validated in the tropics, where there is a higher biodiversity and, hence, the number of species potentially affected is also higher. Therefore, there is a need to validate whether the threshold concentrations (HC₅) derived from the SSDs constructed with laboratory toxicity data are also protective for tropical freshwater ecosystems.

Daam et al. (2008, 2009) compared threshold concentrations derived from (semi-) field experiments in the temperate region and in the tropical region of Thailand for two pesticides: the insecticide chlorpyrifos and the fungicide carbendazim. It was concluded that temperate threshold concentrations show a sufficient protection level for tropical freshwater ecosystems. However, because our knowledge of tropical freshwater ecotoxicology is still very limited, it was recommended to conduct more model ecosystem studies with a wider range of pesticides on a larger geographical scale within the tropical zone. Accordingly, toxicity studies conducted under (semi-)field conditions, taking into account large assemblages of freshwater indigenous species and studying the actual fate and behavior of pesticides in the field, should be carried out in tropical areas such as the Amazon, where the intensive application of pesticides might pose a potential risk for the structure and sustainability of freshwater ecosystems.

Conclusions

Amazonian and temperate fish and freshwater arthropods show a similar sensitivity pattern to the insecticide parathion-methyl. It implies that the toxicity data calculated for temperate species (i.e., data from the US or European

pesticide registration process) can be used in the derivation of safe environmental concentrations of parathion-methyl in the Amazon. However, the conclusions of this study should be treated with caution because they are based on a limited number of laboratory acute toxicity data. Further studies should be aimed at the validation of these conclusions with a wider array of acute and chronic toxicity data and studying the actual fate and effects of this compound under (semi-)field conditions in the Amazon.

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