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Toxicities of glyphosate- and cypermethrin-based pesticides are antagonic in the tenspotted livebearer fish (*Cnesterodon decemmaculatus*)

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HIGHLIGHTS

- Mixtures of glyphosate and cypermethrin are antagonic in *Cnesterodon decemmaculatus*.
- Antagonism is due to an inhibition of cypermethrin toxicity by glyphosate.
- Results in fish are opposite to tadpoles which show synergy.

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ABSTRACT

Although pesticide contamination of surface waters normally occurs in the form of mixtures, the toxicity and interactions displayed by such mixtures have been little characterized until now. The present study examined the interactions prevailing in equitoxic and non-equitoxic binary mixtures of formulations of glyphosate (Glifoglex®) and cypermethrin (Glextrin®) to the tenspotted livebearer (*Cnesterodon decemmaculatus*), a widely distributed South American fish. The following 96 h-LC50s were obtained when pesticide formulations were tested individually: Glifoglex® 41.4 and 53 mg ae glyphosate/L; Glextrin® 1.89 and 2.60 μg cypermethrin/L. Equitoxic and non-equitoxic mixtures were significantly antagonic in all combinations tested. The magnitude of the antagonism (factor by which toxicity differed from concentration addition) varied between 1.37 and 3.09 times in the different non-equitoxic mixtures tested. Antagonism was due to a strong inhibition of cypermethrin toxicity by the glyphosate formulation, the toxicity of the cypermethrin-based pesticide being almost completely overridden by the glyphosate formulation. Results obtained in the current study with fish are radically opposite to those previously observed in tadpoles where synergy was observed when Glifoglex® and Glextrin® were present in mixtures (Brodeur et al., 2014).

1. Introduction

Around the globe, freshwater systems from agricultural regions are contaminated by mixtures of diverse pesticides (Konstantinou et al., 2006; Gilliom, 2007; Giroux and Pelletier, 2012; Moreira et al., 2012; Bereswill et al., 2013; Bonansea et al., 2013; De Geronimo et al., 2014; Smiley et al., 2014). Pesticides enter aquatic ecosystems via diffuse entry pathways such as spray drift, accidental overspray, runoff and drainage (Schäfer et al., 2011).

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Pesticide contamination of surface waters is most commonly associated to a cocktail of substances rather than a single compound because several active ingredients are commonly applied simultaneously to crops and a variety of crop types are normally intermingled in agricultural basins (Lydy et al., 2004; Verro et al., 2009).

The wide adoption of genetically-modified (GM) crops is a predominant feature of modern agriculture. The global surface area planted with GM crops has steadily increased at a rate of 3–4% (or 6.3 million hectares) for the last 19 years to reach 181.5 million hectares in 2014 (James, 2014). The main genetic trait incorporated to crops is the tolerance to the broad-spectrum herbicide glyphosate, which allows producers to apply this herbicide to control weeds without harming the crop (Duke and Powles, 2008). Glyphosate-resistant soybean, corn, cotton and canola constitute the four principal GM crops, with the United States, Brazil and Argentina leading the production (James, 2014). As a consequence of their wide use on transgenic crops, glyphosate-based herbicides are currently the world's bestselling herbicides and the backbone of modern no-till agriculture (Duke and Powles, 2008).

Cypermethrin is a non-systematic pyrethroid insecticide with a wide range of applications that is used in nearly all agricultural crops, as well as in homes and gardens. Cypermethrin and other pyrethroids are a fundamental part of insect pest control in GM soybean crops and in many other row crops such as canola, sunflower, sorghum, barley and wheat (Bolsa de Cereales, 2014; Yang and Suh, 2015). As both glyphosate and cypermethrin are amongst the most used agricultural pest products on a global level, it is likely that these molecules frequently occur simultaneously in aquatic system. Tank mixtures and combined applications of the two pesticides have furthermore been reported in the pampa region of Argentina, mainly in the spring, when recently emerged crops require both herbicide and insecticide control (local producers and agronomist, pers. comm.).

Although pesticide mixtures are most representative of real-life exposures, the quantification and characterization of their toxicity and interactions has received limited attention until now. Overall, past studies have resulted in a spectrum of interactions depending on the mode of toxic action and chemical properties of the pesticides examined (Lydy et al., 2004). In the case of binary mixtures of glyphosate and cypermethrin, previous work (Brodeur et al., 2014) demonstrated the presence of a strong synergy between the two pesticides when looking at acute toxicity in tadpoles of the common South American toad (*Rhinella arenarum*). To expand on these previous results and better understand the risk posed by pesticide mixtures on South American aquatic ecosystems, the present study examined the toxicity of equitoxic and non-equitoxic binary mixtures of glyphosate and cypermethrin formulations to a widely distributed South American aquatic vertebrate: the tenspotted livebearer fish (*Cnesterodon decemmaculatus*).

2. Materials and methods

2.1. Fish collection and husbandry

Tenspotted livebearer (*Cnesterodon decemmaculatus*) were captured with dip nets in Juan Blanco River, Department of Magdalena, Buenos Aires Province, Argentina (35° 8′30.14″ S; 57° 26′28.08″ O). Lands surrounding the sampling site are exclusively dedicated to low-intensity pasture and lie within the limits of "Parque Costero del sur" a UNESCO Biosphere Reserve of 265 km^2 created in 1997.

Fish were placed in tanks containing aerated river water upon capture and were transported within a few hours to laboratory installations at the "Instituto Nacional de Tecnología Agropecuaria". In the laboratory, fish were kept in a climate-controlled room at 20 ± 2 °C and 16:8 h light:dark photoperiod for at least 21-days before being used in the experiments. Characteristics of the well water used for both holding and testing fall within accepted guidelines for fish testing (OECD, 1992) and are presented in Table 1. Animals were fed commercial fish flakes daily throughout the acclimation period. Fish used in the experiments measured 17.8 ± 5 mm and weighed 0.05 ± 0.03 g.

2.2. Test substances

The commercial products Glifoglex® and Glextrin® were used for testing glyphosate and cypermethrin, respectively. Glifoglex® is a commercial formulation of glyphosate (*N*-[phosphonomethyl] glycine; CAS No. 1071-83-6) containing 48% of glyphosate in the form of isopropylamine salt which corresponds to 36% of glyphosate acid equivalent (ae). Glextrin® is a commercial emulsionable formulation of cypermethrin ([Cyano-(3-phenoxyphenyl)methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate; CAS No. 52315-07-8), containing 25% of a mixture of cis and trans isomers of cypermethrin (cis 40–50%). Both pesticide formulations included proprietary surfactants, additives or emulsifiers of undisclosed molecular nature.

2.3. Preparation of test solutions

New stock solutions were prepared each time test solutions were replaced. The diluent used to prepare stock solutions was the culture water described above. Nominal concentrations of stock solutions were 3600 mg ae/L for glyphosate and 12.5 mg/L for cypermethrin. Test solutions were prepared by diluting the adequate volume of stock solution in culture water.

Glyphosate concentration of stock solutions was verified through UPLC Waters Acquity (Ultra Performance Liquid Chromatography) with SQD detector (single quadrupole mass detector) using Hypercarb 2.1 × 100 mm 5 μm column, 1% Acetic acid in

Table 1
Chemical characteristics of well water used in holding tanks, control groups and treatments.

Total dissolved solids	560 mg/L
Total alkalinity (as $CaCO_3$)	260 mg/L
Hardness	90 mg/L
pH	8.2
Chlorides (Cl^-)	6 mg/L
Sulphates (SO_4^{2-})	2 mg/L
Iron (Fe)	<0.05 mg/L
Calcium	18 mg/L
Conductivity	806 μs/cm
Ammonium (NH_4^+)	0.05 mg/L
Nitrite (NO_2^-)	<0.1 mg/L
Nitrate (NO_3^-)	3 mg/L
Fluorides (F^-)	0.74 mg/L
Arsenic (As)	<0.01 mg/L
Lead (Pb)	<0.05 mg/L
Silver (Ag)	<0.05 mg/L
Copper (Cu)	<0.1 mg/L
Zinc (Zn)	<0.5 mg/L
Residual active chlorine	0 mg/L
Mercury (Hg)	<0.001 mg/L
Chrome (Cr)	<0.01 mg/L
Cyanide (CN^-)	<0.01 mg/L
Cadmium (Cd)	<0.005 mg/L
Vanadium	<0.05 mg/L
Calcium	18 mg/L
Aluminium	<0.01 mg/L
Bromate	<0.001 mg/L
Nickel (Ni)	<0.001 mg/L
Selenium	<0.001 mg/L

water: MeOH, at the following gradient; (95:5)–(95:5) 0–2 min, (95:5)–(0:100) 2–5 min, (100:0)–(95:5) 5–6 min, (95:5) 6–10 min as the mobile phase. The selected ion monitoring (SIM) mode was used in quantification analysis. The mass-spectrometer acquisition settings were: ESI negative, quantifier ion 168 m/z and ion 150 m/z for confirmation, retention time and abundance of the confirmation ion relative to that of quantification ion were used as identification criteria. Limit of detection was 1.30 μg/L.

Cypermethrin concentration of stock solutions was verified through Perkin Elmer Clarus 600 Gas Chromatograph equipped with single quadrupole mass detector (MS). Sample volumes of 1.0 μL were injected into the programmable split/splitless injector, in splitless mode with the split outlet opened after 1.5 min with injector port temperature at 250 °C. The capillary column used was DB-5MS (30 m × 0.25 mm I.D.; 0.25 μm film thickness) (Agilent Technologies). The helium carrier gas was programmed with a constant flow of 30 ml/min. The oven-temperature program was initially set at 80 °C with no hold and ramped to 280 °C at 15 °C/min with a hold of 5 min. The selected ion monitoring (SIM) mode was used in quantification analysis. The mass-spectrometer acquisition settings were: electron-impact ionization 70 eV, quantifier ion 181 m/z and ion 165 m/z for confirmation, retention time and abundance of the confirmation ion relative to that of quantification ion were used as identification criteria. Limit of detection was 1.00 μg/L.

2.4. Experimental protocols

Toxicity tests were conducted over two successive sets of experiments respectively named Trial 1 and Trial 2. Trial 1 included individual toxicity tests for both pesticides and a test of equitoxic mixtures. Trial 2 included a repetition of individual toxicity tests for both pesticides, a repetition of the equitoxic mixtures test and a test of non-equitoxic mixtures. Equitoxic and non-equitoxic mixture tests from Trial 1 and Trial 2 used the 96 h-LC50 values obtained in the corresponding individual toxicity tests as the value of 1 toxic unit (TU).

2.5. Individual acute toxicity of glyphosate and cypermethrin

Concentrations for definitive testing were determined from range-finding tests. Final nominal concentrations tested were as follows: glyphosate 34, 38, 42, 46, 50, 54, 58 mg ae/L, and cypermethrin 0.125, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00 and 16.00 μg/L. The experimental design also included a control group exposed to culture water only. Four replicates were performed for every concentration tested. In every replicate, ten fish were placed in tanks of 27 × 18 × 14 cm containing 4 L of culture water with or without (controls) test chemicals. Temperature was maintained at 20 ± 2 °C throughout the exposure, which lasted 96 h. Seventy-five percent of test solutions (3 L) were replaced once after 48 h of exposure. Dead fish were removed and survival was evaluated daily.

2.6. Acute toxicity of equitoxic and non-equitoxic binary mixtures of glyphosate and cypermethrin

Mixture toxicity was evaluated according to the protocols described by Brodeur et al. (2014) in which the concentration causing 50% mortality (LC50) is considered one TU. For equitoxic mixtures, pesticides were combined in equal proportions to create mixtures in which the sum of TU of both components equaled: 0.5, 1, 1.5, 2, 2.5 and 3 TU.

For non-equitoxic mixtures, four series of experiments were conducted. In the first two series, the concentration of glyphosate was fixed at 0.33 TU (series 1) or 0.66 TU (series 2) in all the treatment groups while the concentration of cypermethrin was 0.1, 0.5, 1, 2 or 3 TU. Conversely, in series 3 and 4, cypermethrin concentration was set at 0.33 and 0.66 TU, respectively, while the concentration of glyphosate was either 0.1, 0.5, 0.75, 1, 1.25, 1.5 or 2 TU. Selected test concentrations were defined following preliminary tests. For both equitoxic and non-equitoxic mixtures, a TU-response relationship was developed and used to calculate the TU value of the mixture that caused 50% of mortality (TU50).

2.7. Data analysis

Concentrations of pesticides and TU of the mixtures resulting in the mortality of 50% of individuals were calculated by fitting a four-parameter logistic regression equation to the survival data with setting bottom at 0% and top at 100%. LogLC50s obtained in the two trials conducted with a same pesticide formulations were compared using a sum-of-squares F test. These analyses were realized using GraphPad Prism software version 5.03.

The “Additivity Zone” of equitoxic mixtures, which represents the range of sums of TU equivalent to 1 when taking into account the 95% C.I. of LC50s of individual pesticides was calculated as follows:

Low limit = 50% of low 95% C.I of cypermethrin LC50 in TU + 50% of low 95% C.I. of glyphosate LC50 in TU
High limit = 50% of high 95% C.I. of cypermethrin LC50 in TU + 50% of high 95% C.I. of glyphosate LC50 in TU

For non-equitoxic mixtures, the factor by which the amount of counterpart pesticide was increased compared to concentration addition (CA) was calculated by dividing TU of counterpart pesticide experimentally needed to reach 50% mortality by TU theoretically necessary according to CA (i.e. 0.33 or 0.66). Similarly, the factor by which the toxicity of the mixture was reduced compared to CA was calculated by summing the TU of cypermethrin and glyphosate experimentally needed to reach 50% mortality and dividing the sum by 1, the expected value of sum of TU according to CA.

3. Results

3.1. Pesticide concentrations in stock solutions

Actual concentrations of stock solutions were consistent with nominal concentrations and averaged (mean ± standard error, n = 4), 3795.3 ± 88.1 mg ae/L in the case of glyphosate and 15.1 ± 0.54 mg/L for cypermethrin.

3.2. Individual acute toxicity of glyphosate and cypermethrin formulations

Survival of fish in the control groups was between 98 and 100%. Dose-response curves obtained in both trials with glyphosate- and cypermethrin-based pesticides are illustrated in Figs. 1 and 2, respectively, while concentrations causing mortality of 50% of tenspotted livebearer after 24, 48, 72 and 96 h of exposure are shown in Tables 2 and 3. Dose-response patterns differed considerably between glyphosate and cypermethrin formulations, the dose-response curve of the glyphosate product being clearly steeper than that of the cypermethrin formulation (Figs. 1 and 2). This difference in the time-course of lethality induction is highlighted by the fact that the ratio 24 h:96 h LC50s is close to 1 for glyphosate (indicating that toxicity basically reaches its maximum after 24 h of exposure) whereas it is of around 5 for cypermethrin (Tables 2 and 3).

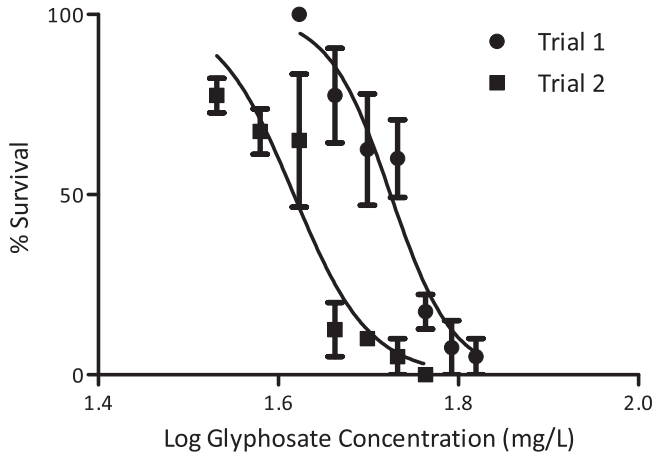

Fig. 1. Dose-response curves of survival in function of glyphosate concentration in *C. decemmaculatus* after 96 h of exposure in both of the trials conducted.

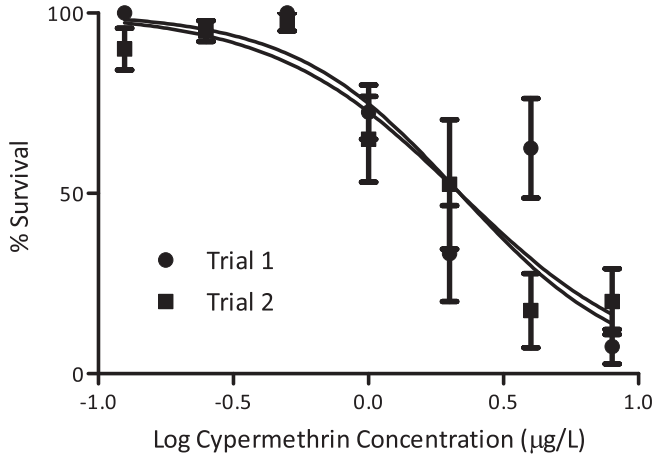

Fig. 2. Dose-response curves of survival in function of cypermethrin concentration in *C. decemmaculatus* after 96 h of exposure in both of the trials conducted.

formulation.

3.3. Acute toxicity of equitoxic and non-equitoxic binary mixtures of glyphosate and cypermethrin formulations

Sums of TU of equitoxic mixtures causing 50% mortality (TU50) are shown in Fig. 3 for both trials. Survival of animals from the control groups was between 98 and 100%. As both TU50 fell outside and to the right of the additivity zone, results clearly demonstrate that glyphosate and cypermethrin formulations are antagonistic, as they are less toxic than the sum of their individual toxicity when combined in equitoxic mixtures.

The isobologram shown in Fig. 4 illustrates results obtained for the four series of non-equitoxic mixtures tested. The diagonal isobole linking the values on the X and Y axes with values of 1 TU is the line of concentration addition (Fig. 4). The fact that all combinations of glyphosate and cypermethrin causing 50% of mortality lie above and to the right of the additivity line indicates the occurrence of antagonistic interactions in all non-equitoxic mixtures tested (Warne, 2003). This means that, as in the case of equitoxic mixtures, glyphosate and cypermethrin formulations are less toxic than the sum of their individual toxicity when combined in non-equitoxic mixtures.

Table 4 details the composition of non-equitoxic mixtures causing 50% mortality and describes the factors by which antagonism reduced the toxicity of the different mixtures compared to CA. This table highlights the fact that cypermethrin toxicity was completely antagonized by the presence of glyphosate, as it was necessary to add 1 TU of glyphosate in order to reach 50% mortality (indicating that glyphosate accounted for all the mortality) when TU of cypermethrin were fixed at either 0.33 or 0.66. In a similar manner, cypermethrin toxicity was inhibited when glyphosate was present in the mixture at either 0.33 or 0.66 TU, as it was necessary to add respectively 2.5 and 7 times more cypermethrin than predicted by CA in order to reach 50% mortality (Table 4). Overall, the magnitude of the antagonism observed in the different series of non-equitoxic mixtures was as follows: Series with 0.66 TU glyphosate fixed > Series with 0.33 TU glyphosate fixed = Series with 0.66 cypermethrin fixed > Series with 0.33 cypermethrin fixed

Table 2
Concentrations of glyphosate (mg/L) causing the mortality of 50% (LC50) of *C. decemmaculatus* after 96 h of exposure in both of the trials conducted. Confidence intervals 95% (CI) are indicated in parenthesis. Glyphosate values are expressed in terms of acid equivalents (ae).

Trial	24 h	48 h	72 h	96 h	Ratio 24 h:96 h
1	56.0 (54.4–57.7)	55.6 (54.1–57.2)	55.5 (54.0–57.0)	53.0 (51.1–55.1)	1.06
2	42.5 (40.6–44.4)	42.0 (40.2–43.9)	41.6 (39.8–43.5)	41.4 (39.6–43.3)	1.03

Table 3
Concentrations of cypermethrin (µg/L) causing the mortality of 50% (LC50) of *C. decemmaculatus* after 96 h of exposure in both of the trials conducted. Confidence intervals 95% (CI) are indicated in parenthesis.

Trial	24 h	48 h	72 h	96 h	Ratio 24 h:96 h
1	14.40 (6.45–32.2)	8.80 (4.93–15.98)	2.66 (1.81–3.88)	2.60 (1.78–3.79)	5.54
2	9.45 (7.97–11.2)	8.61 (7.39–10.0)	1.91 (1.41–2.58)	1.89 (1.36–2.58)	5.0

LC50s obtained in the two trials with cypermethrin were not significantly different at 2.6 and 1.89 µg/L, respectively. For their part, although coherent in terms of general level of toxicity, the LC50s measured for glyphosate were more variable and significantly different at 53 and 41.4 mg/L. Overall, taking into account confidence intervals of LC50s, the cypermethrin formulation was 14.5–28.6 thousand times more toxic than the glyphosate (Table 4).

4. Discussion

Interactions prevailing in mixtures of glyphosate- and cypermethrin-based pesticides were examined in the tenspotted livebearer fish because these pesticides are widely used in row

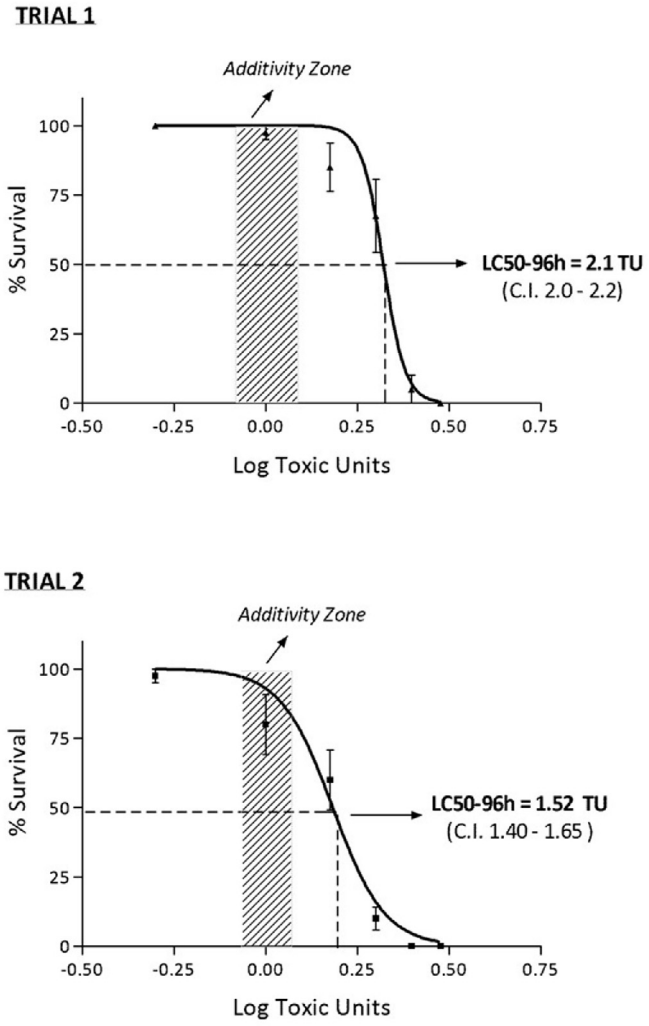

Fig. 3. Dose-response curves of survival in function of the sum of toxic units (TU) of equitoxic mixtures of glyphosate and cypermethrin in *C. decemmaculatus* after 96 h of exposure in both of the trials conducted. The sum of TU of the mixture causing 50% mortality (LC50) is indicated in each graph with 95% confidence intervals (C.I.) in parenthesis. The "Additivity Zone" illustrates the interval of TU within which the LC50 would be placed if the mixture was additive.

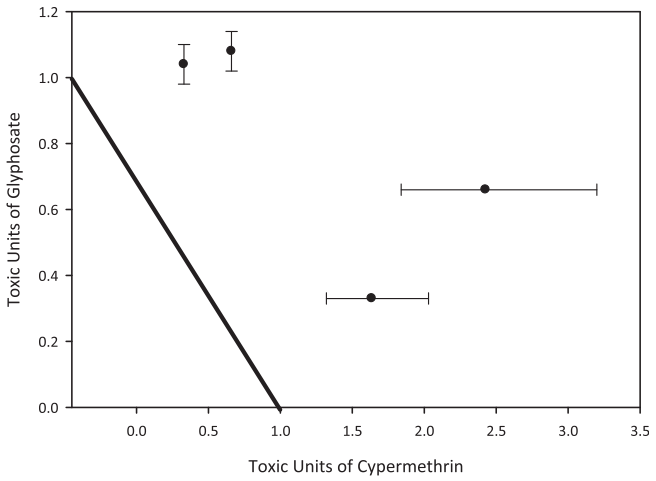

Fig. 4. Isobologram illustrating the composition in toxic units (TU) of non-equitoxic mixtures causing 50% mortality (LC50) in *C. decemmaculatus* after 96 h of exposure in the four series tested. Error bars represent 95% confidence intervals (C.I.). The diagonal isobole linking the values on the Y and X axes with values of 1 TU is the line of concentration addition.

crops in both North and South America, and a strong synergy was previously demonstrated amongst these products in tadpoles of the common South American toad (Brodeur et al., 2014). Before considering pesticide mixtures, it was essential to precisely quantify the individual acute toxicity of cypermethrin and glyphosate formulations tested. For both cypermethrin and glyphosate, the two replicate LC50s obtained were similar, confirming that test conditions and animal acclimation were rigorous and stable. Overall, LC50s determined in the current study were comparable to those available in the literature. In the case of cypermethrin, calculated LC50s were 1.89 and 2.6 μg/L which is coherent with the values of 0.43 and 1.87 μg/L previously reported for *C. decemmaculatus* (Parma et al., 2002; Carriquiriborde et al., 2007) and with LC50 data normally reported for fish species in general, which are typically below 10 μg/L (Stephenson, 1982; Bradbury and Coats, 1989; Viran et al., 2003; Saha and Kaviraj, 2008). For glyphosate, literature data are more variable and depend largely on the product tested and the surfactant employed in the formulation. Technical glyphosate is normally less toxic than formulated products with LC50s > 100 mg/L, while Roundup® formulations are usually the most toxic amongst formulated products with LC50s around 8–15 mg/L (Folmar et al., 1979; Servizi et al., 1987; Morgan and Kiceniuk, 1992; Neškovic et al., 1996; Abdelghani et al., 1997; Osten et al., 2005; Shiogiri et al., 2012; Gholami-Seyedkolaei et al., 2013; Lopes Rocha et al., 2015). With values of 53 and 41.4 mg/L, LC50s obtained for glyphosate in the current study are at intermediate position amongst the 96 h-LC50 values of 15.68, 29 and 91.73 mg/L previously reported for *C. decemmaculatus* (Menéndez Helman et al., 2013; Vera-Candioti et al., 2013).

It is interesting to note that, even if LC50s measured in the current study are coherent with other data from the literature in terms of toxicity, the difference amongst the values are large enough to introduce considerable errors when computing TUs. This observation highlights the need to experimentally determine LC50s for the exact animals and pesticides used before conducing mixture experiments. Also worth of interest is the fact that, while the glyphosate formulation used in both this study and in Brodeur et al. (2014) exhibit similar toxicity in fish and tadpoles, the cypermethrin formulation used in both studies was a hundred times more toxic to fish than tadpoles.

With regards to pesticide mixtures, the current study on fish clearly demonstrated the presence of an antagonic interaction between glyphosate- and cypermethrin-based pesticides, the toxicity of the two products being less than additive in all equitoxic and non-equitoxic mixtures tested. Data obtained from non-equitoxic mixture experiments furthermore demonstrated that the antagonism observed is the result of a strong inhibition of cypermethrin toxicity by the glyphosate formulation, the toxicity of the cypermethrin-based pesticide being almost completely overridden by the glyphosate formulation.

Regarding the toxicity of glyphosate formulations, it is widely established in the literature that the toxicity of these pesticide products depends primarily on the surfactant employed in the formulation rather than on the active ingredient (Folmar et al., 1979; Howe et al., 2004; Moore et al., 2012; Wagner et al., 2013). Surfactants are chemically inert substances that can cause narcosis and interfere with sensitive external tissues such as gill membranes (Connell, 2005). Narcosis refers to the general depression of biological activity causing lethargy, unconsciousness and an overall depression in respiratory-cardiovascular activity that may eventually lead to death (Veith and Broderius, 1990). In fish, surfactants

Table 4
Composition in toxic units (TU) of non-equitoxic mixtures causing 50% mortality (LC50) in *C. decemmaculatus* after 96 h of exposure in the four series tested and factors by which these mixtures differ from concentration addition (CA). Confidence intervals 95% (CI) are indicated in parenthesis.

TU of pesticide set fixed	TU of counterpart pesticide needed to reach 50% mortality	Factor by which amount of counterpart pesticide is increased compared to CA	Factor by which toxicity of mixture is reduced compared to CA
Cypermethrin 0.33 TU	1.04 (0.98–1.10)	1.58 (1.49–1.67)	1.37 (1.31–1.43)
Cypermethrin 0.66 TU	1.08 (1.02–1.14)	3.27 (3.09–3.45)	1.74 (1.68–1.8)
Glyphosate 0.33 TU	1.64 (1.32–2.03)	2.48 (2.00–3.08)	1.97 (1.65–2.36)
Glyphosate 0.66 TU	2.43 (1.84–3.20)	7.36 (5.57–9.70)	3.09 (2.05–3.86)

may also cause death by asphyxia due to the swelling of gill lamellae and associated changes in membrane permeability (Granmo and Kollberg, 1976).

Typical features of surfactant toxicity include the presence of a steep dose-response curve and the fact that lethality normally occurs within 24 h (Wong et al., 1997; Smith et al., 2004). These classic features of the toxic response to surfactants and the presence of gill damage are frequently observed in fish studies examining the toxicity of glyphosate-based pesticides, as a result of the important role of surfactants in the toxicity of these products (Folmar et al., 1979; Shiogiri et al., 2012; Gholami-Seyedkolaei et al., 2013; Vera-Candioti et al., 2013; Lopes Rocha et al., 2015). The present study was no different in that aspect, the strong influence of surfactants in the toxicity of the glyphosate-based pesticide being clearly highlighted by the steepness of the dose-response curve and the fact that the value of the 24 h:96 h LC50 ratio was equal to 1.

It is likely that the fast time-course of lethality induction by the glyphosate pesticide is, indirectly, responsible for the inhibition of cypermethrin toxicity by the glyphosate formulation and the resulting antagonism between the two pesticide products. Indeed, as the glyphosate formulation caused death within 24 h, its action prevailed over cypermethrin toxicity which produced death more gradually over the 96 h of exposure. Alternatively, another possible cause of antagonism is at the level of the gills where glyphosate formulations and surfactants in general are known to induce damage and swelling (Granmo and Kollberg, 1976; Shiogiri et al., 2012; Braz-Mota et al., 2015; Lopes Rocha et al., 2015) which may eventually limit cypermethrin uptake normally occurring at this site (Polat et al., 2002).

Results obtained in the current study with fish are radically opposite to those previously observed in tadpoles. Indeed, while the exact same glyphosate- and cypermethrin-based pesticides showed synergism in a previous study with toad tadpoles (Brodeur et al., 2014), an antagonic interaction was observed in the current study on fish. Explaining the physiological or toxicological reasons for this important difference in response between the two animal groups is difficult based on current knowledge. A possible explanation could be that the glyphosate formulation actuated slightly more gradually in tadpoles than in fish, the 24 h:96 h LC50 ratios observed in our experiments with tadpoles being 1.13 and >1.3 (Brodeur et al., 2014). Although slightly higher than those observed in the current study on fish, these ratios are still very close to 1 and it is unlikely that they are the sole explanation for the difference in response observed between fish and tadpoles. In fact, a number of examples can be found in the literature where surfactants and glyphosate formulations produced 24 h:96 h LC50 ratios equal to 1 in tadpoles (Mann and Bidwell, 2001; Howe et al., 2004; Lajmanovich et al., 2010), although Lajmanovich et al. (2003) did observe a ratio of 1.8 in *Scinax nasicus*.

Further studies will be required before the results of this study and the previous one on tadpoles (Brodeur et al., 2014) can be explained from a mechanistic point of view. The findings reported in the two studies are however of immediate interest from a regulatory point of view as agencies around the globe normally use fish as a model to estimate toxicity to all aquatic vertebrates, including amphibians. However, the current study on a fish, in conjunction with our previous study on an amphibian (Brodeur et al., 2014), demonstrated that, in the case of mixtures of cypermethrin- and glyphosate-based pesticide, the responses of the two animal groups are opposite.

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