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Organ distribution of pesticide active ingredient v formulation

Bioaccumulation and Distribution Behavior of Endosulfan on a Cichlid Fish: Differences Between Exposure to the Active Ingredient and a Commercial Formulation.

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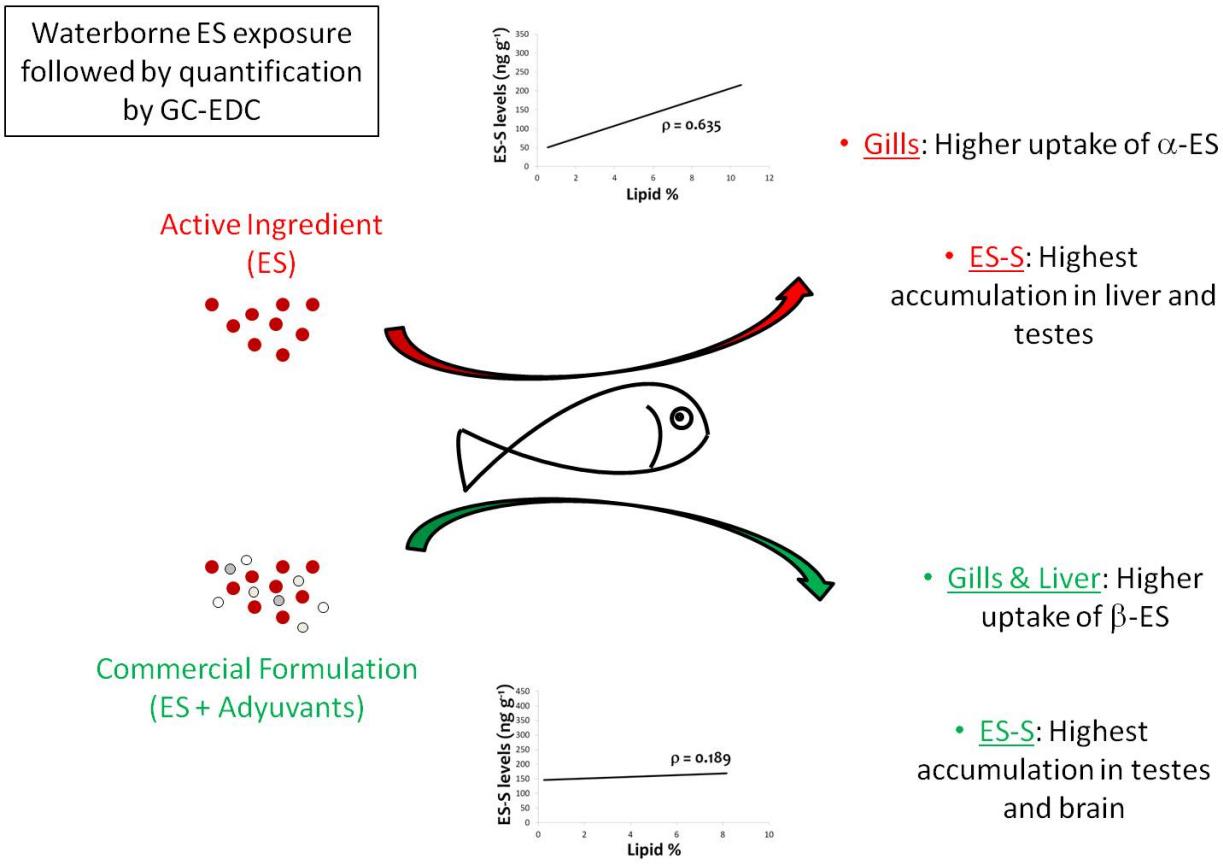
Abstract: Persistent organic pollutants reach aquatic ecosystems during application and can bioconcentrate/biomagnificate due to their lipophilic nature. Toxicological studies focus almost exclusively on the active ingredients (AIs) of pesticides, instead of commercial formulations (CFs), whose toxicity can differ due to non-specified ingredients. The intensive use of endosulfan (ES) as a wide range insecticide over the last few decades make it one of the most frequently detected contaminants in the aquatic environment, even after it has been restricted worldwide. The aim of the present study was to evaluate the bioaccumulation and organ distribution of waterborne ES in the freshwater fish *Cichlasoma dimerus*, comparing between the AI and a CF. Males were exposed to 0.7 μg/L ES for two weeks. ES was quantified (GC-ECD) in liver, testes, gills, brain and muscle. Results suggest rapid metabolism of α-ES and β-ES isomers to ES sulfate (ES-S) in tissues. Isomers levels were highest in gills, indicative of recent uptake. ES-S levels were highest in liver and testes for the AI, and testes and brain for the CF. For the AI, ES-S levels showed a positive correlation with organ-lipid %. No correlation was evident for CF indicating that the presence of adjuvants alters ES distribution, as gills and liver showed a higher uptake and mobilization of β-ES. These differences in organ distribution may alter tissue-specific toxicity, therefore additives cannot be considered inactive even if non-toxic.

Graphical Abstract



Keywords: Bioaccumulation, biotransformation, toxicokinetics, pesticides, freshwater toxicology, persistent organic pollutants.

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INTRODUCTION

Agricultural pesticides can reach aquatic ecosystems during application through air drift, runoff and/or leaching. Once they reach water bodies, the distribution and concentration of toxicants in the different environmental compartments will be dependent on both their physicochemical properties and the environmental parameters that determine the degree of transport, transformation, adsorption and/or bioaccumulation by the aquatic biota (van der Oost et al. 2003). Due to their lipophilic nature, persistent organic pollutants (POPs), including organochlorine pesticides, are able to bioconcentrate and biomagnify through the food chain (Singh and Singh 2008). In addition to the chemical characteristics of the toxicant, organ distribution and transformation of pesticides within an organism can be influenced by body size, lipid content and age of individuals (Spacie et al. 1995; Ondarza et al. 2011, 2014). The longer the half-life of a toxicant in the body, the greater the risk of chronic effects due to the toxicant. Lipophilic compounds require biotransformation in order to be detoxified and/or excreted (Ferreira et al. 2006).

Toxicological studies focus almost exclusively on the active ingredients (AIs) of pesticides, even though the use of pesticide commercial formulations (CFs) by agricultural workers and urban sprayers accounts for thousands of intoxications in developing countries (Litchfield 2005; Sapbamrer 2018). Toxicity of AIs and CFs can differ due to additive or synergistic effects of non-specified “inert” or “inactive” ingredients -adjuvants and additives used to increase solubility, dispersal, persistence and/or uptake. These “inactive” ingredients, whose identity is generally not disclosed,

function to improve the overall effectiveness of the pesticide and usually constitute the majority of the pesticide formulation's volume (Mullin et al. 2015). The toxicity of the AI can either over or underestimate the toxicity of CFs (Pereira et al. 2009; Mesnage et al. 2014). For instance, CFs of the herbicide triclopyr have been shown to exhibit higher genotoxicity in fish than their AI counterpart (Guilherme et al. 2015). Evidence of oxidative stress and cytotoxicity was stronger for a glyphosate formulation than for its AI alone, in a human cell line (Chaufan et al. 2014). In lady beetles, toxicity of CFs of the herbicides 2,4-dichlorophenoxyacetic acid and dicamba were largely driven by the inactive ingredients (Freydier and Lundgren 2016). The nonionic surfactant R-11 present in insecticide formulations has been shown to synergize the acute toxicity of the AIs spinosad and imidacloprid on aquatic crustaceans (Deardorff and Stark 2009; Chen et al. 2010).

The organochlorine pesticide endosulfan (ES) has been restricted or banned worldwide after its inclusion in the list of Persistent Organic Pollutants by the Stockholm Convention (UNEP 2011). However, its intensive use over the last few decades as a wide range insecticide in crops of high commercial value like soy, tobacco, cotton, maize and coffee, makes this pesticide one of the most frequently detected contaminants in the aquatic environment in Argentina (Commendatore et al. 2018). Endosulfan is currently detected in aquatic environments worldwide, including in remote locations due to long range transport, being one of the most abundant pesticides in Antarctica and the Arctic (Vorkamp and Rigét 2014; Jantunen et al. 2015; Luek et al. 2017; Rimondino et al. 2018). The pesticide mixture contains a 7:3 ratio of two isomers, α- and β-ES, which differ in their physical-chemical properties (Rice et al. 1997). Endosulfan isomers have

been detected in surface and groundwater in concentrations ranging from 0.01 to 2.5 µg/L (Dalvie et al. 2003; Leong et al. 2007; Ballesteros et al. 2014) as well as in bivalves and fish at relatively high concentrations (Lanfranchi et al. 2006; Barni et al. 2014; Ondarza et al. 2014; Polder et al. 2014). Both isomers can be converted to ES-sulfate (ES-S) by biotic transformation, being α-ES converted more readily to ES-S (Weber et al. 2010). All 3 compounds exhibit high acute toxicity to aquatic organisms, acting as reproductive disruptors in fish (Chakrabarty et al. 2012; Senthilkumaran 2015; Islam et al. 2017).

As few studies have analyzed the difference in bioaccumulation of AIs between exposure to the AI alone or as part of a CF, the aim of the present study was to evaluate bioaccumulation and organ distribution of waterborne ES after acute exposure, comparing between the active ingredient and a commercial formulation using adult males of the freshwater fish *Cichlasoma dimerus*. This species, native to the Paraguay River and most of the Paraná River basins, including some heavily agricultural areas, is a representative perciform of the La Plata River basin, considered an amenable model for laboratory studies, as it acclimates easily to captivity and has been successfully used in ecotoxicological testing (Da Cuña et al. 2011, 2013; Genovese et al. 2014; Piazza et al. 2015).

MATERIALS AND METHODS

Animals

Adult fish of the native freshwater species *C. dimerus* were captured in Esteros del Riachuelo, Corrientes, Argentina (27º35’S 58º45’W). Fish were held in 100 L well

aerated aquaria with external filtration and a layer of gravel on the bottom, with filtered tap water at 25 ± 1 °C, pH 7.3, and 14:10 h photoperiod. They were allowed to acclimatize to laboratory conditions for a month prior to experimentation. During the acclimatization period fish were fed daily with pelleted commercial food (Tetra food® sticks). All experiments were conducted in accordance to international standards on animal welfare (National Research Council 2011) as well as local guidelines (CICUAL, FCEN, UBA).

Exposure experiments

Twelve male fish (mean weight ± SD = 24.5 ± 3.6 g; mean standard length ± SD = 8.1 ± 0.6 cm) were individually and randomly transferred to 10 L aquaria devoid of ornamentations. Once fish were accustomed to this new environment for one week, they were exposed to a nominal concentration of 0.7 μg/L ES, either as the active ingredient alone (AI; 94.99% purity, technical grade, 70:30 α:β stereoisomers mixture, SENASA) or ES in a commercial formulation (CF; Zebra Ciagro® 35%), for two weeks under semi-static conditions with 4 individuals per test group. A solvent control (acetone) was run simultaneously. Steady-state internal levels of ES have been found to be achieved after 7 days in zebrafish (Toledo and Jonsson 1992). A stock solution of each ES was prepared dissolving it in acetone; the necessary volume of stock solution was added to the aquaria to achieve the desired final concentrations (solvent = 0.005% per aquaria); water and the test chemical solutions were renewed daily. Fish were fed daily, an hour before water renewal to minimize chemical adsorption to the pelleted food. At the end of the exposure period, fish were anesthetized with Fish Calmer (active ingredients: acetone,

dimethylketone alpha methyl quinoline, Jungle Laboratories), weighed, measured and euthanized by decapitation. Whole liver and testes were weighed for calculation of the corresponding organ-somatic index as: Organ Weight x 100 / Total Weight. Fulton's condition factor (K) was calculated as: Total Weight x 100 / (Standard Length)^3. Liver, gonad, gills, brain and dorsal skeletal muscle samples were collected and stored at -20 °C until ES extraction.

Chemical analysis

Extraction procedure and ES quantification. Endosulfan (α– and β– isomers) and its metabolite ES-S were extracted according to Metcalfe and Metcalfe (1997) with modifications by Miglioranza et al. (2003). Whole organs (Brain, Testes) or subsamples of organs (450 mg Muscle; 150 mg Liver; 100 mg Gill filaments) were homogenized with anhydrous sodium sulfate and spiked with PCB #103 as surrogate standard. Total lipids and organic compounds were extracted with a 50:50 mixture of dichloromethane and n-hexane (pesticide grade) in a Soxhlet apparatus for 8 h. Lipids were removed from the extracts by gel permeation chromatography using Bio-Beads S-X3 (200-400 mesh) and evaporated to dryness to calculate the sample lipid content. The fraction containing ES was purified by column chromatography with activated silica gel (200 °C for 24 h). Extracts were concentrated to 1 mL and kept at -20 °C prior to gas chromatography analysis. Quantification was performed using a Gas Chromatograph Shimadzu GC-17-A equipped with a ^{63}Ni Electron Capture Detector (GC-ECD) and a capillary column SPB-5 [(5 percent phenyl)-methyl polysiloxane, 30 m x 0.25 mm i.d. x 0.25 μm film thickness; Supelco Inc.]. One microliter was injected into a splitless mode (275 °C) and the detector

was kept at 290 °C. The oven temperature program was: start at 100 °C and hold for 1 min, followed by an increase of 5 °C min^{-1} up to 150 °C, hold for 1 min, then 1.5 °C min^{-1} up to 240 °C, and then 10 °C min^{-1} up to 300 °C for 10 min. Ultra-high purity helium was used as carrier gas (1.5 mL min^{-1}) and nitrogen as make-up gas. Individual standard solutions of ES and PCB #103 (internal standard) were used (Absolute Standards and Ultra Scientific, respectively).

Instrumental blanks were analyzed throughout the procedure to ensure absence of contaminants or sample interference. Results indicated there was no contamination during laboratory handling. The detection limit for both ES isomers and ES-S was 0.09 ng/mL. Endosulfan concentrations were expressed as ng per g of wet weight; lipid content was expressed as percentage. Bioconcentration factors (BCFs) were calculated for each organ analyzed as the ratio between the total concentration of ES in the organ (both isomers and ES-S; ng/kg) and the concentration of ES in water (ng/L) (0.65 +/- 0.06 μg/L).

Statistical analysis

Statistical analyses were carried out using Statistica 7.0 (StatSoft Inc., 2004). Data are presented as mean ± SE and the statistical significance was set at $p < 0.05$. Differences in contaminant levels between organs were tested using a Friedman ANOVA for multiple dependent samples. Differences between experimental conditions were tested using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons. Spearman's correlation coefficients were calculated between lipid percentage and contaminant levels.

RESULTS

Morphometric parameters and body indexes of male *C. dimerus* after exposure are summarized in Table 1. Fish exposed to the CF were significantly lighter with a smaller hepatosomatic index (HIS) than other groups. No differences were observed for standard length, gonadosomatic index (GSI) and condition factor K.

Concentrations of ES in organs of exposed fish showed detectable levels of α-ES in gills > brain > muscle of fish exposed to the AI, whereas levels were below detection limits for liver and testes. On the other hand, fish exposed to the CF, α-ES was below the detection level in all organs, except for gills and liver of one fish (Figure 1A). For β-ES, levels in fish exposed to the AI were gills > brain > muscle > liver, whereas levels for exposure to the CF were liver > gills > brain > muscle. No detectable β-ES levels were measured for testes (Figure 1B). High levels of ES-S were detected in all organs of fish exposed to ES, either to the AI or the CF, with highest levels found in liver and lowest levels in muscle. In decreasing order, ES-S levels were liver > testes > brain > gills > muscle upon AI exposure, and liver > brain > testes > gills > muscle for CF exposure (Figure 1C). In control fish, no detectable levels of either α-ES or β-ES were found, however low levels of ES-S were detected in gills and liver.

On average, lipid content was highest for testis (4.7 ± 1.0 %) and liver (4.1 ± 1.0 %), followed by brain (3.5 ± 0.5 %) and gills (1.8 ± 0.7 %); muscle lipid levels were the lowest (0.7 ± 0.3 %). High interindividual variability in lipid content was observed for testes and liver. When analyzing by exposure condition, muscle of fish exposed to the AI

had a significant higher lipid % than control animals (Table 2). There was a positive monotonic correlation between lipid % and ES-S levels in fish exposed to the AI (Spearman's coefficient 0.635; p = 0.02; Figure 2A). No monotonic correlation was observed for the CF (Spearman coefficient 0.189; p = 0.50; Figure 2B).

The BCFs calculated for total ES (α-ES + β-ES + ES-S) for the organs analyzed are presented in Table 3. As expected, fish exposed to solvent alone (CT) showed the lowest BCF values, only exhibiting low values in gills and liver due to the presence of ES-S. Muscle BCFs were the lowest of all organs when fish were exposed to either the AI or the CF. For both exposure conditions, liver BCFs were the highest of all organs analyzed. In brain and liver, BCFs differed significantly from the three organs with lowest values, muscle, gills and testes.

DISCUSSION

Organ bioaccumulation of toxicants is the outcome of the balance between absorption, distribution, biotransformation and elimination (Lehman-McKeeman 2013). As fish were exposed to a stereo-isomers mixture of ES, the presence of either isomer in fish organs can be explained by uptake from water, whereas ES-S would only be the result of biotic transformation of parental isomers. On average, β-ES values were lower than α-ES, as was to be expected considering that technical grade ratio of α– and β– isomers was 7:3. However, for those organs which showed detectable values of both isomers, ratio values deviated from the isomers ratio in exposure solution, indicative of differential uptake and/or metabolism by fish. When exposed to the AI, the ratio was higher than 7:3 (2.33), suggesting increased uptake of α-ES and/or increased metabolism

of β-ES. The former is the more likely case, considering that hydrolysis of β-ES is higher in aqueous solutions and that this isomer is less susceptible to biotransformation to ES-S (Walse et al. 2003; Weber et al. 2010). For the CF, the α:β isomer ratio was lower than the expected in gills and liver, which could be explained by increased metabolism of α-ES to ES-S or, as they favor stability and penetration of active ingredients into cell membranes (Contardo-Jara et al. 2009), a more likely explanation would be higher uptake of β-ES due to the presence of adjuvants in the CF. In plant cells, adjuvants act increasing uptake of agrochemicals. For instance, surfactants and alcohols increase glyphosate and 2,4-D mobility across cell membranes, respectively (Riechers et al. 1994; Foy and Pritchard 1996). Transdermal penetration of herbicides in mice is also increased for CFs compared to AIs (Brand and Mueller 2002). The metabolite ES-S exhibited greater levels than parent isomers for both the AI and CF, and was detected in all organs studied, signaling that isomers have a relatively short half-life in fish tissues and, in the case of α-ES, are readily biotransformed and redistributed throughout the animal.

Uptake of waterborne chemicals in fish is achieved through the skin, gills and/or gastrointestinal tract. Since fish were exposed to waterborne ES, gills would have been the main route of absorption, as dermal uptake is a minor route in larger fish (Lien and McKim 1993). Fish gills are specialized for efficient exchange of respiratory gases by having a thin epithelium, a large surface area and counter-current blood and water flows; these same traits also favor xenobiotics uptake directly from water (Kleinow et al. 2008). Absorption through the gills is related to the relative hydrophobicity of the compound, reaching maximum uptake rates for chemicals with octanol-water partition coefficients

(log K_{ow}) between 3 and 6 (McKim et al. 1985). As ES isomers have log K_{ow} within this range (3.83 for α-ES and 3.62 for β-ES; Hansch et al. 1995), gills constituted one of the organs, together with brain, with the highest levels of both isomers in fish exposed to the AI. Even though gills were still one of the sites of highest accumulation of ES isomers following exposure to the CF, values were lower than those detected for the AI, which could suggest that adjuvants cause ES isomers to more readily move across the gill epithelium and distribute to other organs by way of systemic circulation. Surfactants, such as POEA included in glyphosate formulations, have been shown to affect permeability of cell membranes and therefore increase absorption capacity of biologically active agents (Benachour and Seralini 2009).

Xenobiotics transport across one or more biological membranes is necessary to reach target organs and exert deleterious effects. Once absorbed, toxicants may distribute throughout the body based on their physicochemical characteristics, their concentration gradient, the relative affinity for different tissue components and the blood flow to different organs (Lehman-McKeeman 2013). The liver, as one of the main detoxification sites, is among the organs with highest blood perfusion rates in fish (Schultz et al. 1999), and also showed one of the highest lipid percentages of the organs analyzed in *C. dimerus*. This likely determined the accumulation of high levels of β-ES when fish were exposed to the CF; alternatively α-ES levels were considerably lower and neither isomer was detected in liver after exposure to the AI. As biotransformation of β-ES to ES-S is less prevalent (Walse et al. 2003; Weber et al. 2010), the results found for the exposure to the CF suggest that the presence of coadjuvants would have contributed to the uptake of this isomer in liver following the exposure period.

The blood-brain barrier, formed by complex tight junctions between brain capillary endothelial cells, maintains homeostasis of the brain microenvironment essential for normal function (Lee et al. 2004; Ohtsuki et al. 2004). Chemicals that are able to pass through this barrier have the potential to be neurotoxic (Harry et al. 1997). Being that the main mechanism of action of ES in target species is the antagonism of GABA receptors (ATSDR 2000) and that both isomers are able to permeate through the blood-brain barrier of vertebrates (Chan et al. 2006), β-ES was detected in brain upon exposure to both the AI and the CF and α-ES was present in the former. As was the case for liver, other constituents of the CF seem to increase organ uptake or retention of β-ES leading to its detection in brain following exposure. Since α-ES is more efficient in disrupting blood-brain barrier integrity (Chan et al. 2006), its absence in brain following exposure to the CF again points to a differential action of adjuvants on both isomers.

The liver plays a pivotal role in the excretion of xenobiotics by producing polar metabolites that can be readily excreted in bile (Gingerich et al. 1977; Carriquiriborde et al. 2012). Biotransformation of xenobiotics occurs in the liver by non-synthetic alteration (oxidation, reduction or hydrolysis) and conjugation by phase I and phase II enzymes, respectively (Commandeur et al. 1995). Oxidation of ES results in ES-S, which is more persistent and toxic than ES isomers (US EPA 2007). This metabolite can be further hydrolyzed, particularly to endosulfan diol which is the main detoxification and excretion product in fish (Rao and Murty 1982). As an intermediary metabolite, ES-S could be found in high levels in most of the organs analyzed, either as a result of local production or distribution according to its relative tissue affinities, in particular its lipid solubility (Kleinow et al. 2008). Organs with high lipid content may act as repositories for

lipophilic compounds, so chemical levels may correlate to lipids percentage in different organs. When fish were exposed to the AI, there was a positive correlation between lipid % and ES-S levels, being testes, liver and brain the organs with highest accumulation of the metabolite, whereas gills and muscle showed the lowest levels. Contrarily, upon CF exposure no correlation was evident and in this case only liver and brain showed high ES-S concentrations, as levels in testes, gills and muscle were not statistically significant from each other. These results suggest that when exposed to the AI, the resulting ES-S formed partitions according to its Log K_{ow} value (3.66; Hansch et al. 1995) and its lipid solubility. Alternatively, the presence of adjuvants in the CF alters this distribution, particularly for testes. A stronger correlation with lipid content could be of importance during sexual reproduction, as in sexually mature fish lipid can mobilize from fat storages to gonads to provide energy for spawning behaviors (Jobling et al. 1998). A CF of ES was shown to trigger precocious ovarian development and upregulation of aromatase activity and 17β-estradiol levels in juvenile catfish (*Clarias batrachus*; Chakrabarty et al. 2012). In males of this species, the same CF did not alter testosterone levels, even though expression of several steroidogenic enzymes was decreased (Rajakumar et al. 2012). A different CF caused lower testicular testosterone levels following exposure in a cyprinid fish (*Cyprinion watsoni*; Islam et al. 2017). In the present study species, *C. dimerus*, the AI was capable of disrupting sex steroid production stimulated by gonadotropins in both testes and ovaries (Da Cuña et al. 2016). These varying effects on steroidogenesis could be the result of differential ES levels in gonads through exposure to different CFs or the AI, though species-specific responses to ES could also be a factor.

A measure of the extent to which a chemical is able to bioaccumulate is the bioconcentration factor (BCF) (Arnot and Gobas 2006), the ratio of the concentration of a substance in an organism (absorbed through respiratory and dermal surfaces) relative to the concentration in the environment. In liver and gills of control animals, the BCFs calculated due to trace amounts of ES-S were likely the result of past exposure to ES in the original environment from where fish were obtained, even though all animals were maintained at least two weeks in clean water prior to the onset of experimentation. When exposed to waterborne ES, BCFs were above 100 for all organs analyzed except for muscle, being significantly higher in liver and brain, but no significant differences were observed between exposure to the AI or the CF. For other agrochemicals, such as glyphosate, higher accumulation tendency (thus higher BCF) was determined for CF than for the AI (Contardo-Jara et al. 2009). In our study, edible tissues (muscle) showed the lowest levels of ES isomers, ES-S and BCFs, for both the AI and the CF. Similar results were observed when *Mugil cephalus* adults were exposed to ES in sea water for 28 days, with only trace amounts of α-ES and β-ES detected in edible tissues (Schimmel *et al*. 1977). Alternatively, in *Catla catla*, muscle was the main storage site of ES isomers of all the organs analyzed following exposure (Rao 1989).

As chemicals with higher affinity for organic matter are more likely to bioaccumulate in fatty tissues, potential to bioaccumulate can be assessed by a combination of BCF and log K_{ow}. Substances with BCF values below 100 or log K_{ow} below 3 are generally considered not to bioaccumulate, whereas those with a BCF over 1,000 or a log K_{ow} over 4 are likely to bioaccumulate significantly (Schäfer et al. 2015). Those chemicals whose BCF falls between 100 and 1,000 and its log K_{ow} between 3 and

4 are thought to have the potential to bioaccumulate. Both the log Kow for both ES isomers and ES-S, as well as the BCFs calculated in the present study for *C. dimerus* organs, save muscle, fall within this latter range. For other fish species, ES BCF values cover a wide range, from below 100 (4-60 for gills and liver of *Astyanax fasciatus* and *Pimelodus maculatus*, Paulino et al. 2014), between 100 and 1,000 (258-272 for *Catostomus commersoni*, 314 for *Carassius auratus*; Goebel et al. 1982), to above 1,000 (2650 for whole-body *Danio rerio*, Toledo and Jonsson, 1992; 2755 for whole-body *M. cephalus*, Schimmel et al. 1977). These different bioaccumulation patterns could be attributed to species differences in absorption, biotransformation and excretion and also to sex, age and physiological conditions.

In summary, results suggest a rapid metabolization of ES isomers to ES-S in fish tissues, since isomers were present in highest amounts in gills, indicative of recent uptake, and ES-S levels were highest in most organs. Exposure to the AI resulted in ES-S organ distribution positively correlated to the lipid content of organs. For the CF distribution did not follow this pattern, possibly due to the action of coadjuvants. As toxic effects of pollutants are expressed when distributed to a site of action, these differences in organ distribution of ES between exposure to a CF or the AI alone may alter toxicity. This effect can become more prominent during the spawning season, when fish mobilize fat storages to provide energy for reproductive processes. Based on this evidence, additives used in formulations cannot therefore be considered inactive even when they exhibit no toxicity on their own and CFs as a whole should be assessed when studying the effect of pesticides on non-target biota.

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Data Availability—All data used in the development of this publication can be made available by request (rhdacu@bg.fcen.uba.ar).

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Figure 1: Levels of α-ES (A), β-ES (B) and ES sulfate (ES-S, C) in organ samples from male *Cichlasoma dimerus* exposed to solvent (CT), ES active ingredient (AI) or an ES commercial formulation (CF). B, brain; G, gills; L, liver; M, muscle; T, testes. Different letters denote significant differences between organs for a given compound and exposure condition (Friedman ANOVA; $p < 0.05$).

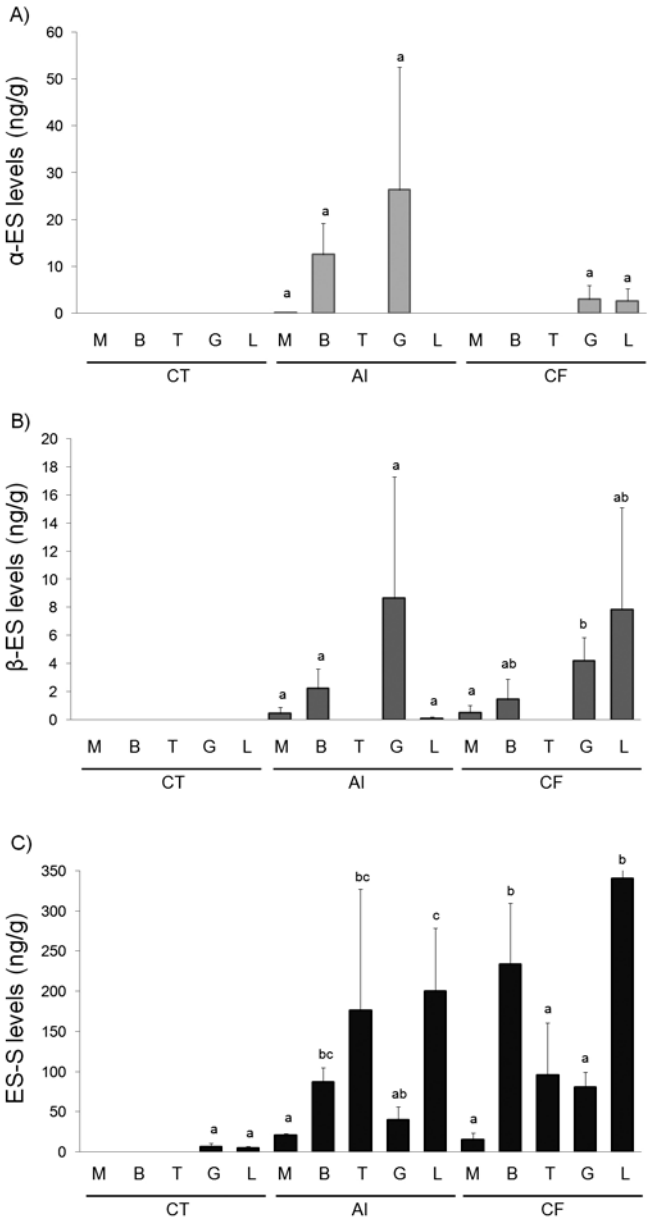
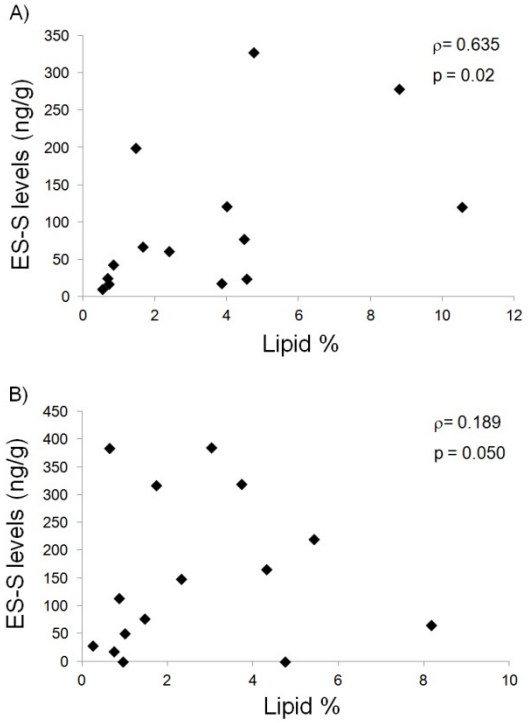


Figure 2: Correlation between lipid percentage of each organ and its ES-S levels for AI (A) or CF (B) exposure. Spearman's correlation coefficient (ρ) was significant for AI and not significant for CF.



Graphical Abstract: Bioaccumulation and organ distribution of endosulfan (ES) isomers and their main metabolite ES-sulfate (ES-S) differed when fish were exposed to the active ingredient alone or to a commercial formulation (CF). These differences in organ distribution may alter tissue-specific toxicity, therefore additives present in CFs cannot be considered inactive even if non-toxic.

Table 1. Morphometric parameters and body indexes of *C. dimerus* following ES exposure[a]

	Body weight (g)	Standard length (cm)	HSI	GSI	K (g/cm^3)
CT	26.9 ± 1.0A	8.5 ± 0.3A	1.79 ± 0.05A	0.048 ± 0.006A	4.41 ± 0.30A
AI	25.8 ± 2.0A	8.3 ± 0.3A	1.79 ± 0.27A	0.071 ± 0.023A	4.47 ± 0.18A
CF	19.9 ± 0.6B	7.7 ± 0.2A	0.81 ± 0.09B	0.052 ± 0.013A	4.72 ± 0.15A

[a]Different letters denote significant differences between exposure conditions for each parameter (One-Way ANOVA, followed by Tukey's test; $p < 0.05$).

AI: Active ingredient; CF: Commercial formulation; CT: Control; GSI: Gonadosomatic index; HSI: Hepatosomatic index; K: Fulton's condition factor.

Table 2. Lipid content (%) of *C. dimerus* organs exposed to waterborne ES[a]

	Muscle	Brain	Testes	Gills	Liver
CT	0.15 ± 0.06A	2.92 ± 1.26B	2.79 ± 0.37B	0.56 ± 0.05AB	4.12 ± 2.37B
AI	1.76 ± 1.05AB*	3.62 ± 0.63AB	4.64 ± 0.10B	1.02 ± 0.34A	6.94 ± 2.78AB
CF	0.65 ± 0.21A	3.21 ± 0.58BC	6.10 ± 1.05B	1.10 ± 0.18AC	2.03 ± 0.91AC

[a]Different letters denote significant differences between organs for a given exposure condition (Friedman ANOVA; $p < 0.05$).

*Denotes significant differences with CT exposure for the same organ (One-Way ANOVA; followed by Tukey's test; $p < 0.05$).

AI: Active ingredient; CF: Commercial formulation; CT: Control.

Table 3. Bioconcentration factors for total ES in different organs of *C. dimerus* after exposure[a]

	Muscle	Brain	Testes	Gills	Liver
CT	n.c.	n.c.	n.c.	18.2 ± 12.4	7.2 ± 2.9
AI	29.8 ± 4.1A	145.1 ± 28.8B	251.3 ± 216.2A	107.2 ± 56.9A*	285.5 ± 113.2B*
CF	33.7 ± 6.3A	335.6 ± 110.5B	204.3 ± 111.0A	125.6 ± 31.7A*	501.2 ± 26.3B*

[a]Different letters denote significant differences between organs for a given exposure condition (Friedman ANOVA; $p < 0.05$).

*Denotes significant differences between exposure condition and control for a given organ (Kruskal Wallis test; followed by multiples comparison; $p < 0.05$).

AI: Active ingredient; CF: Commercial formulation; CT: Control; n.c.: not calculated, ES values below the detection limit.