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Bioconcentrations of herbicides used in sugarcane crops in tilapia (*Oreochromis niloticus)* and the risk for human consumption

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ABSTRACT

The practice of intensive herbicide use in the sugarcane industry has a high risk of compromising the quality of the water and the organisms that live there due to losses through runoff, leaching and other processes. In this work, the dynamics of four herbicides present in three different mixtures were evaluated through their incorporation and elimination in the muscle tissue of tilapia (*Oreochromis niloticus*). The highest mean values of bioconcentration factors were 1.730 for ametryn, 0.891 for tebuthiuron, 0.322 for hexazinone and 4.783 for diuron. Diuron presented the highest risk regarding the consumption of tilapia fillets by the population. However, considering that the fish would reach maximum levels of diuron when exposed to extremely high concentrations, an individual weighing 70 kg would need to ingest approximately 1.5 kg of this food product to surpass the acceptable daily intake of 0.007 mg kg^{-1} body weight. It was concluded that the risk of injury to the population consuming tilapia fillets from fish exposed to herbicides in water arising from sugarcane activities is very low.

According to the risk estimation performed in this work, which is substantiated by the assumptions of the World Health Organization and the International Life Sciences Institute, there is a low risk of injury to the population consuming tilapia fillets from fish exposed to water containing herbicides in concentrations arising from sugarcane activities. However, as the risk was estimated from laboratory conditions, caution should be taken where herbicide applications are carried out with high frequency near water bodies, as the consumption of fish from these areas is quite common.

1. Introduction

Sugarcane is one of the most important commodities in the world, with a production of 635 million tons estimated in 2018/2019 and harvested from an estimated 8.66 million hectares in Brazil (CONAB, 2018). To increase crop productivity, herbicides are widely used in Brazil. In this context, sugarcane producers use the herbicide ametryn (AMT) in combination with tebuthiuron (TBUT) and the commercial product Velpar K WG®, a mixture of diuron (DIU) and hexazinone (HZN).

These chemicals are used in the following proportions: AMT + TBUT = 2.0 + 1.2 kg active ingredient (a.i.) ha^{-1}; (DIU + HZN)+TBUT = 2.0 + 1.2 kg a.i. ha^{-1}; (DIU + HZN)+TBUT + AMT = 2.0 + 1.2 + 1.5 kg a.i. ha^{-1} (Moura and Jonsson, 2016).

In Brazil, the use of a mixture of pesticides is allowed if it is prescribed by an agronomist. Such use is an advantage for the farmer, since it amplifies the spectrum of action of the herbicides. However, as previously demonstrated for pesticide mixtures, their use can amplify the toxic effects of each component on nontarget organisms (Laetz et al., 2009; Prestes et al., 2011; Silva et al., 2015).

Once present in the environment, herbicides can accumulate in the soil, undergo leaching, be transported via surface runoff to water bodies and enter the tissues of aquatic organisms. With regard to this last phenomenon, Uno et al. (2001) detected, at a relatively high frequency, herbicide residues of thiobencarb, molinate, and chlornitrofen in organs of the bivalve *Anodonta woodiana* exposed to effluents from rice plantations.

Residues of triazine herbicides such as atrazine, simazine, propazine, terbutrine, prometrone, prometrine and ametryn were found in the muscles and livers of birds and mammals and in fish from the Baltic Sea (Reindl et al., 2015).

The pyridate and fluazifop-P-butyl herbicides were detected in fillet and viscera samples of the *Prochilodus costatus*, a fish collected from the

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São Francisco River, Brazil (Oliveira et al., 2015).

Herbicides have been previously detected in Brazilian water bodies (Santos et al., 2013), which implies the potential for accumulation in aquatic organisms, some of them at concentrations greater than the current maximum permissible levels (Mattos et al., 2002; Pinheiro et al., 2010; Moreira et al., 2012). Tebuthiuron and ametryn were found to be present in São Paulo State waters at levels of 0.01 and 0.29 μg L^{-1}, respectively (Monteiro et al., 2014). In a region with extensive sugarcane cultivation, Armas et al. (2007) detected the presence of hexazinone, glyphosate, clomazone and triazine herbicides (ametryn, atrazine, and simazine) in surface waters and sediments of the subbasin of the Corumbataí River (São Paulo State, Brazil).

There is growing interest in predicting the accumulation of xenobiotics in aquatic biota from experiments conducted under laboratory conditions to establish values of ecotoxicological endpoints. In this context, a parameter relating the concentration of an herbicide in an aquatic animal to its concentration in the water at steady state is the bioconcentration factor (BCF). In situations where a steady state is not attained, the BCF can be estimated by two-phase regression models or kinetics models (Jonsson and Toledo, 1993; Andreu-Sánchez et al., 2012) to calculate the uptake and depuration constants. Therefore, the BCF is an estimate of a chemical's propensity to accumulate in aquatic animals. Additionally, herbicide-exposed animals can depurate in a clean environment, and time series analyses of the residues are valuable in determining how long biota will retain a chemical.

Therefore, for a fish hypothetically exposed to a given concentration, the BCF allows the estimation of daily fish consumption so that the acceptable daily intake of the herbicide (ADI) is not exceeded. Bioconcentration factor values also help establish safe limits of herbicide concentrations in the aquatic environment so that fish tissue concentrations do not introduce risk to the consumer (Spacie and Hamelink, 1985).

Because of the importance of fish for human consumption due to their distribution in the aquatic environment, these animals have typically been used for bioaccumulation assessments. Toxicity data from studies with fish are therefore needed to estimate the human and environmental risks caused by the use of herbicides, individually or in the form of mixtures.

According to Call et al. (1987), Tucker et al. (2003) and the USDA (2013), the low levels of tissue accumulation and the rapid elimination indicate that organic herbicides do not tend to accumulate in fish and cause problems due to their residues, and these are rapidly lost. A very low BCF, equivalent to 2, was determined for atrazine in the muscle of *Coregonus fera* fish exposed to a concentration of 0.253 mg L^{-1} (Gunkel and Streit, 1980).

According to the USDA (2013), of the eleven herbicides proposed for the control of invasive plants in the State of Oregon (USA), the majority do not accumulate; only three presented low accumulation degrees: chlorsulfuron (BCF = 1.5), sethoxydim (BCF = 7) and sulfometuron methyl (BCF = 7).

However, while most herbicides have relatively low BCF values, some herbicides may accumulate in fish tissue in a greater proportion, despite being eliminated in more than 80% of their residues within 24 h. This is the case for propanil that gave BCF values of 69 and 111 in the fish *Pimephales promelas* exposed at concentrations of 0.34 and 5.1 μg L^{-1}, respectively (Call et al., 1983).

The herbicide benthiocarb bioconcentrates 160 to 625 times more in several species of fish in relation to its concentration in the water. BCF values equivalent to 286, 163 and 150 were calculated for molinate, symetrin and simazine from fish exposed to river waters of Japan containing these active ingredients (Tsuda, 2011).

In previous work, we reported the oxidative stress and histopathological effects due the exposure of tilapia (*Oreochromis niloticus*) to sublethal concentrations of the herbicide mixtures used in sugarcane crops in São Paulo State, Brazil (Jonsson et al., 2017). This fish is widely distributed in the Brazilian territory and created in the most diverse production systems due to its relevant commercial interest (Vicente et al., 2014).

Tilapia represents 51.7% of Brazilian fish production, with 357 thousand tons harvested in 2017, placing Brazil among the top four global producers (Associação Brasileira da Piscicultura, 2018).

According to Adolfsson-Erici et al. (2012), if a second chemical in the mixture either inhibits or induces enzymes involved in the metabolism of the test chemical, a change in the BCF determination may result. This means that the bioconcentration processes may be altered by the presence of another chemical for which metabolism is expected to be the dominant elimination mechanism. Therefore, these arguments led us to carry out studies with simultaneous exposure to the herbicides since they are applied in mixtures in sugarcane crops and consequently can be found simultaneously in water bodies. Thus, if any interference between the herbicides occurs as described by the authors above, we would obtain more realistic BCF values to predict risks.

According to the considerations above, in the present work, the degree of accumulation of the herbicides (AMT, TBUT, HZN and DIU) was evaluated by determining the value of BCF resulting from the incorporation and elimination of muscle tissues from tilapia submitted to sublethal doses. The data allowed us to estimate the risk of fish fillet consumption by the population when these organisms are exposed to these herbicides, which are used in large amounts.

2. Materials and methods

2.1. Test materials

The tests were performed using mixtures of herbicide formulations based on tebuthiuron (TBUT - Combine 500 SC®; concentrated suspension; 500 g L^{-1}); ametryn (AMT - Gesapax 500®, concentrated suspension; 500 g L^{-1}) and a commercial mixture of diuron (DIU) and hexazinone (HZN) (Velpar K®), which consists of dispersible granules containing DIU (468 g kg^{-1}) and HZN (132 g kg^{-1}). All formulations were obtained from local suppliers. Analytical standards of purity >98% were purchased from Dr. Ehrenstorfer (Augsburg, Germany).

2.2. Test organisms and exposure

The fish characteristics, handling and exposure conditions were previously described (Jonsson et al., 2017). Fish with an average weight of 33.48 ± 6.15 g and an average length of 9.98 ± 0.56 cm were obtained from a commercial fishery in São Paulo State (Brazil) and placed in polyethylene tanks (115 L usable volume). The experimental systems were constantly aerated and installed in a climate-controlled room with a photoperiod of 16 h/8 h (light/dark) and a temperature of 26 ± 2 °C. The water used had the following characteristics: pH = 7.7; dissolved oxygen = 6.2 mg L^{-1}; electrical conductivity = 3.8 mS cm^{-1}; and total hardness = 53.6 mg L^{-1} $CaCO_3$.

After an initial acclimation of at least one week, the fish were exposed to the control treatment and two sublethal test concentrations, C1 and C2, of each mixture (Table 1). This corresponds, respectively, to 1/100 and 1/10 of the average lethal concentration of exposure for 96 h (LC_{50}-96 h) previously determined (Moura and Jonsson, 2016). The LC_{50}-96 h values determined for each mixture in previous tests (Moura and Jonsson, 2016) were as follows: AMT + TBUT 10.76 mg L^{-1}, (DIU + HZN)+ TBUT 43.09 mg L^{-1}, and (DIU + HZN)+TBUT + AMT 11.90 mg L^{-1}.

Table 1
Nominal concentrations used in the assimilation phase of the herbicide mixtures by tilapia (*Oreochromis niloticus*).

Mixture	Treatment (mg L^{-1})	
	C1	C2
(DIU + HZN) + TBUT	0.431	4.310
AMT + TBUT	0.107	1.076
AMT + (DIU + HZN)+TBUT	0.119	1.190

These values represent the total amounts of herbicides in each mixture. The amounts of the active ingredients in the mixtures tested were proportional to the quantities used in sugarcane plantations, as described previously (Introduction).

Therefore, at the highest exposure level (1/10 LC_{50}-96 h), the nominal concentrations (mg L^{-1}) in the mixtures were 0.67 + 0.40 (AMT + TBUT), 2.70 + 1.60 ((DIU + HZN)+TBUT), and 0.51 + 0.30 + 0.38 ((DIU + HZN)+TBUT + AMT). At the lowest exposure level (1/100 LC_{50}-96 h), the concentrations were 1/10 of these values.

Table 1 shows the 1/100 LC_{50}-96 h (C1) and the 1/10 LC_{50}-96 h (C2) values that represent the sum of the herbicide amounts in each mixture. These amounts were used to prepare the nominal exposure concentrations.

From each tank (15 fish per tank) containing the test concentration (in duplicate), three fish were sampled at 7 and 14 days of exposure (assimilation phase). After the end of this phase, the remaining fish were kept for 14 days in xenobiotic-free water, and the samplings were carried out on the 7th and the last day (14th day) of this depuration phase.

Animals were fed twice a day *ad libitum* with commercial food, and the media in the tanks were totally renewed every two days.

At each sampling period, the animals were anesthetized with benzocaine diluted in water and euthanized at the spinal medulla. The muscular tissue was collected and stored at -80 °C until it was processed for residue analysis. The procedures used in this study were authorized by the Animal Experimentation Ethics Committee of Embrapa Environment (Registration N° 002/2012).

2.3. Method of analysis of herbicides in fish and test solutions

2.3.1. Tilapia fillet

The analytical method for the determination of herbicide multiresidue in tilapia (*O. niloticus*) muscle was optimized and validated. The concentration of the herbicides was determined by weighing 5.0 g of the sample and using an established QuEChERS method (Anastassiades et al., 2003) with some adaptations as follows: a high-performance disperser (Ultraturrax) was used to grind the sample (5 g) in a 50-mL polypropylene conical centrifuge tube with 10 mL of acetonitrile. The mixture was vortexed for 30 s. Then, 4 g of anhydrous $MgSO_4$, 1 g NaCl, 0.5 g of disodium hydrogen citrate sesquihydrate and 1 g of trisodium citrate were added, and the mixture was vortexed again for 30 s. After centrifugation at 10,000 rpm for 7 min (at 5 °C), an aliquot of the supernatant (7 mL) was transferred to another centrifuge tube containing 750 mg anhydrous magnesium sulfate and 125 mg of PSA sorbent. The tube was vortexed for 30 s and then centrifuged again at 10,000 rpm for 7 min at 5 °C. An aliquot of the extract (2 mL) was submitted to evaporation under nitrogen flow and resuspended in 1.0 mL of mobile phase. Finally, the extract was transferred to a vial for injection into the LC–MS/MS.

Chromatographic separations were performed using a Varian 1200L LC-MS/MS instrument equipped with an electrospray source in positive mode and a triple quadrupole type analyzer (QqQ). The acquisition was performed in MRM (multiple reaction monitoring) mode, and the protonated ion $[M + H]^+$ was selected as the precursor ion for each herbicide, namely, AMT, DIU, HZN and TBUT, with two transitions, one for quantification and one for confirmation. In the chromatographic separation, a Polaris 3 C18 A (5 μm, 2.0 mm ID, 150 mm, Agilent) column was used. The flow rate was set at 0.25 mL min^{-1}, and a linear gradient from 60 to 95% organic phase over 10 min was used (Vilhena et al., 2013). The extract was resuspended in 0.5 mL mobile phase (60:40, v/v, methanol/0.1% aqueous formic acid), filtered with a 0.45 μm filter and analyzed. The validated method presented a high correlation coefficient ($R \geq 0.99$) and a quantification limit (LQ) of 0.00125 μg g^{-1} for AMT, DIU, HZN and TBUT. The recoveries were performed at two levels of analyte concentration (LQ and 2 x LQ). The range of recoveries required is between 70 and 120%, and the coefficient of variation (CV) is less than 20%, according to the SANTE/11813/2017 guidelines (European Commission, 2018).

2.3.2. Water

Samples of water were collected from the tanks after the preparation of the test solutions and prior to water renewal. The samples were added directly to the vial and then injected into an ultra-performance liquid chromatograph coupled to a triple quadrupole mass spectrometer with electrospray ionization (UPLC-ESI-MS/MS), model Quattro Premier XE, Waters. The chromatographic separation employed an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 mm ID, 50 mm) maintained at a temperature of 40 °C. The mass spectrometer was operated in MRM mode with positive ion electrospray ionization. The limit of quantification (LQ) was 0.010 mg L^{-1} for all herbicides with recoveries between 107.7 and 127.3% and a coefficient of variation <3% (Jonsson et al., 2017).

2.4. BCF determination

In order to certify the steady state attainment, Student's t-test was used to verify that the herbicide concentrations in the muscle at day 7 and at day 14 were not significantly different (Stephen et al., 2010). This test was performed by the use of the program Statgraphics Centurion XVII (Version 17.1.04), where the level of significance was set at $p < 0.05$ (StatPoint Technologies, 2014). Subsequently, the determination of the BCF for each exposure concentration (C1 and C2) was estimated by the ratio (Eq. 1) of the concentration of the residue in the fish muscle (7 and 14 days) of the assimilation phase at steady state (*Cfss*) to the concentration in the test solution (*Cwss*) at steady state (OECD, 2012).

$$BCF = C_{fss} / Cwss \quad (1)$$

For situations in which the steady state was not reached because a significant difference in the residues between day 7 and day 14 was verified, the BCF was determined by the ratio of the uptake rate constant (k_1) to the elimination rate constant (k_2) (Eq. 2).

$$BCF = k_1 / k_2 \quad (2)$$

The constant k_1 was calculated by Eq. (3) (Spacie and Hamelink, 1985)

$$k_1 = ((\Delta C_f / \Delta t) + k_2 C_f) / Cw \quad (3)$$

where C_f is the concentration of the herbicide in fish at the uptake phase at time *t*; *Cw* is the concentration of the herbicide in the test solution at time *t*; $\Delta C_f/\Delta t$ is the tangent of the assimilation curve; and k_2 is the elimination constant. This last parameter was estimated by the natural logarithm transformed concentration (*ln* concentration) vs. depuration time, where the slope of the regression line is an estimate of k_2 (OECD, 2012) that was determined by the program described above.

3. Results and discussion

3.1. Evaluation of the herbicide bioconcentration

3.1.1. Residues in the test solutions

The remaining concentrations of the active ingredients in the test solutions (C1 and C2) during the assimilation phase (7, 11 and 14 days) were determined. The means of the residues before the renewal of the test solutions and after renewal were considered (Tables 2, 3, and 4). These values represent the "actual" concentrations of residues determined in the water to which the fish were exposed and were used to calculate the ratio of the concentration of residues in the fish and the residue concentration in the water during the assimilation phase.

The concentrations determined were lower than the nominal concentrations, which may have been due to the absorption of the herbicide in the test vessels, as previously described by Jonsson et al. (2017). This may have been facilitated by the other components of the formulation (Topp and Smith, 1992; Wheelock et al., 2005).

Table 2
Mean values (±standard deviation) of herbicide residues in the muscle of *O. niloticus* (mg kg^{-1}) and in the test solution (mg L^{-1}) and bioconcentration factors (L kg^{-1}) from the exposure to two nominal concentrations (C1 and C2) of AMT + TBUT for 14 days.

	Concentration			
	Fish (C1)	Fish (C2)	Test solution (C1)	Test solution (C2)
AMT	0.067 (0.027)	0.662 (0.125)	0.055 (0.032)	0.296 (0.078)
TBUT	0.028 (0.009)	0.230 (0.043)	0.027 (0.006)	0.307 (0.007)
	BCF			
	(C1)	(C2)	Mean	C.I. 95%*
AMT	1.229 (1.215)	2.231 (1.007)	1.730 (1.111)	0.190–3.227
TBUT	1.033 (0.530)	0.748 (0.156)	0.890 (0.343)	0.415–1.366

* Confidence interval 95%.

Table 3
Mean values (±standard deviation) of herbicide residues in the muscle of *O. niloticus* (mg kg^{-1}) and in the test solution (mg L^{-1}) and bioconcentration factors (L kg^{-1}) from the exposure to two nominal concentrations (C1 and C2) of (DIU + HEX) + TBUT for 14 days.

	Concentration			
	Fish (C1)	Fish (C2)	Test solution (C1)	Test solution (C2)
HEX	0.011 (0.003)	0.123 (0.024)	0.045 (0.008)	0.473 (0.133)
DIU	0.323 (0.111)	3.244 (0.802)	0.109 (0.037)	1.001 (0.292)
TBUT	0.089 (0.023)	0.938 (0.135)	0.116 (0.030)	0.955 (0.268)
	BCF			
	(C1)	(C2)	Mean	C.I. 95%*
HEX	0.249 (0.122)	0.385**,[a] (0.067)	0.322 (0.094)	0.191–0.512
DIU	4.454**,[b] (1.796)	5.113**,[c] (1.547)	4.783 (1.671)	2.467–7.099
TBUT	0.769 (0.399)	0.982 (0.416)	0.876 (0.408)	0.310–1.441

* Confidence interval 95%.
** BCF value calculated by the relation $k_1/k2$.
[a] $k_1 = 0.268$ L kg^{-1} d^{-1}; $k_2 = 0.698$ d^{-1}.
[b] $k_1 = 3.742$ L kg^{-1} d^{-1}; $k_2 = 0.840$ d^{-1}.
[c] $k_1 = 6.031$ L kg^{-1} d^{-1}; $k_2 = 1.180$ d^{-1}.

3.1.2. Residues in tissues

Tables 2, 3, and 4 present the herbicide levels in the tilapia muscle exposed to the three herbicide mixtures for 14 days at two sublethal concentrations (C1 and C2). The tables also present mean BCF values calculated from the bioconcentration processes in test solutions C1 and C2.

Table 4
Mean values (±standard deviation) of herbicide residues in the muscle of *O. niloticus* (mg kg^{-1}) and in the test solution (mg L^{-1}) and bioconcentration factors (L kg^{-1}) from the exposure to two nominal concentrations (C1 and C2) of (DIU + HEX) + TBUT + AMT for 14 days.

	Concentration			
	Fish (C1)	Fish (C2)	Test solution (C1)	Test solution (C2)
AMT	0.030 (0.005)	0.296 (0.067)	0.038 (0.000)	0.179 (0.054)
TBUT	0.015 (0.003)	0.176 (0.029)	0.023 (0.012)	0.225 (0.058)
HEX	0.002 (0.000)	0.019 (0.003)	0.036 (0.034)	0.093 (0.016)
DIU	0.028 (0.009)	0.427 (0.198)	0.025 (0.009)	0.189 (0.062)
	BCF			
	C1	C2	Mean	C.I. 95%*
AMT	0.797 (0.141)	1.657 (0.877)	1.227 (0.509)	0.522–1.933
TBUT	0.662 (0.458)	0.782 (0.328)	0.722 (0.393)	0.177–1.267
HEX	0.049 (0.058)	0.200 (0.071)	0.124 (0.064)	0.035–0.214
DIU	1.104 (0.769)	2.261 (1.788)	1.683 (1.278)	n.d.**. – 3.454

* Confidence interval 95%.
** n.d.: non-determinate.

In general, considering the three mixtures evaluated, the sequential order of BCF values for the four compounds was DIU > AMT > TBUT > HEX. Therefore, DIU was the herbicide that presented the highest BCF values, reaching bioconcentration in the fish approximately 4.5 times higher than the mean concentration. The BCF values found for this herbicide in the present study (BCF = 4.4 and 1.6) are close to those reported by Call et al. (1987) in the fish *Pimephales promelas* (BCF = 2.0). However, according to Fojut et al. (2010), a BCF value of 290 in the *Gambusia affinis* fish was recorded.

In relation to the other herbicides in terms of order of magnitude, the literature assigns BCF values higher than those calculated in the present study: 4 to 5 for HEX (USDA, 2001); 1.98 for TBUT (USEPA, 1994a); and 33 for AMT (PPDB, 2018). Even so, these values have a low bioconcentration potential in fish tissue and would classify these herbicides as slightly to moderately bioaccumulative (Zagatto, 2006).

In order to compare with our results, the BCF values of the four active ingredients were estimated by the equation logBCF = 0.83 logKow - 1.71, which has a high correlation (r = 0.97) between the BCF in catfish and the coefficient of octanol/water partition (Kow) for a series of organic molecules with pesticide action (Murty, 1986). The BCF values calculated through this equation corresponded to 4.4 for DIU; 2.9 for AMT; 0.6 for TBUT and 0.2 for HEX. This sequence of BCF values corroborates with the sequence of BCF values determined in the present work, as well as the magnitude of these values.

3.2. Herbicides depuration by O. niloticus

The results demonstrated a rapid elimination of the active ingredients from tilapia tissue when exposure to xenobiotics ceases.

DIU and HEX were the only two herbicides that showed quantifiable levels in the elimination period, and this occurred for organisms exposed to the mixture (DIU + HEX) + TBUT, just on the 7th day of the depuration phase. The mean concentrations of DIU for this period were 0.0795 ± 0.0612 (n = 5) and 0.1112 ± 0.0906 (n = 6) mg kg^{-1} for exposure concentrations C1 and C2, respectively. This represented 24.61% and 3.44% of the concentration at the beginning of the depuration period, respectively.

The higher DIU tissue retention time in relation to the other herbicides can be explained by the higher Kow value of 707.94 (Cerdeira et al., 2015) and the lower solubility in water (42 mg L^{-1}) than the other compounds. According to Call et al. (1987), DIU is rapidly eliminated in fish, with 76–84% clearance in a period of 24 h after transfer to water free of the herbicide. After 21 days, approximately 99% is eliminated.

According to Tucker et al. (2003), DIU residues in catfish (*Ictalurus punctatus*) remained below one-half of the permissible maximum limit (2 mg kg^{-1}) in fish fillets after successive application of 9 weekly doses of 0.01 mg L^{-1} in water to control cyanobacteria. No residues were detected after two to four months since the last application, with a detection limit of 0.05 mg kg^{-1}.

With respect to HEX, only three samples had quantifiable levels with mean ± standard deviation equivalent to 0.0023 ± 0.00056 mg kg^{-1} for the exposure concentration C1, which represented 20.35% of the concentration at the beginning of depuration phase. Although this compound was found on the 7th day of clearance, its accumulation in fish tissues is not expected (USEPA, 1994b; USEPA, 1996).

These low levels of tissue accumulation and rapid elimination support the argument that the tendency of organic herbicides does not cause problems associated with residue levels and that the residues are very rapidly lost (Call et al., 1987).

The elimination rate of a compound is often associated with a positive correlation with water solubility, which correlates negatively with Kow and consequently with BCF (Bowman and Sans, 1983; OECD, 2012). HEX is the compound with the greatest solubility in water, which supports its nondetection in tissues, as was observed with AMT and TBUT. However, the presence of HEX in the elimination phase may be associated with its

metabolization or other factors. According to Newman (2013), the latter include transport through the gills, secretion via hepatic and excretion. The same author argues that the elimination process occurs through a combination of physical, chemical and biological mechanisms and that increasing solubility does not necessarily always lead to increased elimination.

The insufficiency of samples that demonstrated quantifiable levels for the 7th and 14th days of purification and the absence of detection of the molecules in the tissue for these periods made it impossible to construct decay curves of the residues as a function of time in order to estimate kinetic parameters of clearance. However, we applied the conservative method proposed by the OECD (2012), where the value of the quantification limit may be used as the lower point in the linear regression *"ln* concentration in tissue vs. depuration time*"*. This value was used for the estimation of *k2*.

3.3. Risk of human consumption of tilapia fillets

According to the World Health Organization (WHO) (UNEP/IPCS, 1999), if a measured bioconcentration factor (BCF) for a fish species is available, this factor can be used to estimate the concentration expected in the fish exposed to a known or a predicted concentration of a substance in water. WHO also notes that this calculation is likely to give an overestimate of the actual fish concentration and that once an estimated concentration in fish has been obtained, this can be used along with fish dietary intake figures to estimate human exposure.

As stated by the International Life Sciences Institute (ILSI), the potential exposure to a xenobiotic is compared to the ADI, denoting the maximum amount of food a person can ingest daily, where the risk is acceptable when the ingestion of the compound is less than the ADI established (Benford, 2000).

Another consideration is that for substances such as herbicides, which have low BCF values, the degree of bioconcentration is similar to the degree of bioaccumulation. The latter takes into account the uptake through food in addition to uptake through water (Stephen et al., 2010).

With respect to the assumptions above, the risk of ingestion of a daily amount of muscle tissue from tilapia exposed to the herbicide mixture was determined based on the hypothetical concentration of the residue that would be reached in that tissue. For this evaluation, the upper limit of the 95% confidence interval of the BCF ($UL_{95\%}$-BCF) was adopted to assume the case of "highest risk". The use of such an upper limit is a common practice in risk assessment that considers the worst-case scenario (WHO, 2008).

In this work, the worst case would be for a person who consumes the fish with the highest herbicide residue, derived from the $UL_{95\%}$-BCF, taken from an aquatic environment with the highest concentration level. This level would be attained with a direct herbicide application over the water body with a given water column.

Data are presented in Table 5 as the estimated fish consumption risk by the population based on the acceptable daily intake (ADI) (Australian Government, 2005) of each herbicide in each mixture. Table 5 also shows the estimated maximum concentration of each herbicide in the aquatic environment (EMC_{water}) according to Peterson and Hulting (2004) and Zagatto (2006), who consider a 2-meter water column for estimation purposes of the risk. Thus, direct herbicide application was considered at the application rate (AR; kg ha^{-1}) over a water column at that depth.

It should be noted that the adopted depth value is similar to the mean depth related to tilapia production in net tanks (Turco et al., 2014; Scorvo Filho et al., 2008).

The expected hypothetical concentration of herbicide residue in fish muscle (Exp C_{fish}) exposed in this situation was calculated by multiplying $UL_{95\%}$-BCF by EMC_{water}. With these data and the ADI value, the maximum daily intake of fish fillet (MDI) was calculated for an adult person (body weight = 70 kg) in order to not exceed the ADI value (Table 5), that is, the safe amount of fish fillet to be consumed without risk to human health considering the daily limit of ingestion of the herbicide according to regulatory toxicological parameters.

The results indicate that the greatest restriction is for the herbicide DIU since the individual would need to consume approximately 1.5 kg of fish per day to reach the ADI value. We should take into account that this estimate was calculated in a maximum risk scenario by the direct application of a dose of the agrochemical. In this situation, the decrease in the herbicide concentration in water by adsorption in the sediment or any kind of compound degradation that would occur in a field situation was not considered.

It should also be considered that the accumulation in tissue obtained in our experimental situation was due to successive exchanges of test solutions, a fact that would most likely not occur in field conditions.

According to Sartori and Amâncio (2012), the fish consumption per person in the Southeast region of Brazil was, on average, 5.4 kg/year. The highest consumption was recorded in the Northern region (38 kg/year), considering that this region is not strictly a sugar cane production area. According to Lopes et al. (2016), the consumption of fish by the Brazilian population is still small, averaging approximately 9 kg/person/year. This quantity is below that recommended by the Food and Agriculture Organization (FAO) (12 kg/person/year). This institution reports (FAO, 2018) that the average national consumption of freshwater fish from catch fishing and freshwater aquaculture is quite low at only 3.95 kg per capita per year. However, the fish consumption resulting from these procedures is close to 150 kg per capita per year in the Amazon region. Even so, such a high value does not attain the 1.47 kg/day of tilapia fillet consumption established in this work for reaching the ADI of DIU.

According to VMK (2008), when the sum of the exposure doses of the individual compounds in the mixture does not exceed the ADI for the most potent compound, there should be no apparent concerns. In this context, our data demonstrate that the sum of the four herbicide doses may not exceed the ADI of DIU (0.007 mg/kg b.w./day), even with a diet highly rich in fish proteins, as mentioned above for the Amazon region.

Table 5
Maximum daily intake (MDI) of tilapia fillet from a hypothetical exposure of fish to herbicide mixtures and risk parameters for human consumption.

Mixture	Herbicide	$UL_{95\%}$-BCF	AR (kg ha^{-1})	EMC_{water} (mg L^{-1})	Exp C_{fish} (mg kg^{-1})	ADI*	MDI**
AMT + TBUT	AMT	3.27	2	0.10	0.327	0.02	4.28
	TBUT	1.37	1.2	0.06	0.082	0.07	59.61
(DIU + HEX) +TBUT	HEX	0.51	0.26	0.013	0.007	0.1	1055.81
	DIU	7.10	0.93	0.047	0.334	0.007	1.47
	TBUT	1.44	1.2	0.06	0.086	0.07	56.71
(DIU + HEX) +TBUT + AMT	AMT	1.93	1.5	0.075	0.145	0.02	9.67
	TBUT	1.27	1.2	0.06	0.076	0.07	64.30
	HEX	0.21	0.26	0.013	0.003	0.1	2564.10
	DIU	3.45	0.93	0.047	0.161	0.007	3.02

$UL_{95\%}$-BCF = upper limit of the 95% confidence interval of the BCF; AR = application rate of the herbicide; EMC_{water} = estimated maximum concentration of the herbicide in the aquatic environment; Exp C_{fish} = expected hypothetical concentration of herbicide residue in fish muscle; ADI = acceptable daily intake of the herbicide.

* mg herbicide per kg body weight per day.

** kg fish fillet per day per 70 kg person.

4. Conclusion

The risk estimation used in the present work was based on a well-known recommended concept of international identities that includes the amount of food that is ingested daily, the maximum amount of xenobiotic that can be ingested without causing an apparent injury to human health and the concentration of the xenobiotic residue in food. With respect to xenobiotic residue in food, the BCF determination allowed for the estimation of the hypothetical concentration in the fish muscle.

It can be concluded that when national and international data of fish consumption are compared with the MDI values calculated in this work, the harm risk by the fish consuming population via herbicide contamination due to sugarcane farming can be considered very low. It should be noted that this consideration is based on results generated from controlled experimental conditions that are not necessarily similar to field conditions, which could influence the compound accumulation pattern. Although this risk estimate is based on a worst-case scenario for the consumption of tilapia exposed to herbicides through water, caution should be taken where herbicide applications are carried out with high frequency near water bodies, as the consumption of fish from these areas is quite common. The intake of other food products that would also contain these herbicides should also be considered.

Likewise, estimating a high per capita consumption based on data from regions that have fish as the main source of protein input indicates that the daily intake of herbicides by this source would contribute little to reaching the ADI values. These facts are justified by low BCF values of herbicides and rapid elimination in tilapia fish verified in the present work, as well reported in the literature for other fish species.

Declarations

Author contribution statement

Claudio Jonsson, Mônica Moura, Vera Ferracini, Lourival Costa Paraiba, Marcia Assalin, Sonia Queiroz: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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