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Acute Toxicity of Agricultural Pesticides to Embryo-Larval and Juvenile African Catfish *Clarias gariepinus*

**P. T. Agbohessi · I. Imorou Toko · A. Houndji ·
V. Gillardin · S. N. M. Mandiki · P. Kestemont**

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Abstract Acute toxicities of Tihan 175 O-TEQ, as well as its active ingredients flubendiamide and spirotetramat, and of Thionex 350 EC (active compound endosulfan) were measured for embryo-larval and juvenile stages of the African catfish *Clarias gariepinus* to assess risks of pesticide use in the cotton basin in Benin (West Africa). For embryo-larval stages, Tihan was more toxic ($LC50_{48h}$ 20 ppm) than Thionex ($LC50_{48h}$ 56 ppm), and flubendiamide was more toxic ($LC50_{48h}$ 2.0 ppm) than spirotetramat ($LC50_{48h}$ 8.44 ppm). All decreased hatching rates. Tihan and spirotetramat disturbed larval swimming coordination; flubendiamide induced tail cleavage. For juvenile fish, Thionex was more toxic ($LC50_{96h}$ 0.22 ppm) than Tihan ($LC50_{96h}$ 8.8 ppm), and flubendiamide ($LC50_{96h}$ 4.7 ppm) was more toxic than spirotetramat ($LC50_{96h}$ 6.0 ppm). Eggs were more resistant than juvenile fish to all tested pesticides except flubendiamide. Although Thionex was more toxic to juvenile fish, replacing Thionex with Tihan may be undesirable for survival of eggs and larvae.

Aquatic ecosystems are the final destination of pesticides used in agricultural production (Gillium 2007; Chao et al. 2009). Since 1960, the Benin Agriculture Ministry (West Africa) has authorized the use of an increasing number of insecticides and other pesticides by cotton producers, but there is no effective regulation to prevent their escape into the wider environment (Agbohessi et al. 2012). Endosulfan causes a range of environmental problems, and several cases of human poisoning have been reported by the Ministry of Health (Mbaye 2008). Consequently, the use of endosulfan has been discouraged in Benin since 2007, and the product Tihan 175 O-TEQ, a mixture of flubendiamide (100 g/L) and spirotetramat (75 g/L), has been recommended to replace it. Despite this prohibition by the Beninese authorities, endosulfan is still used extensively in cotton production. Banikoara territory produces the greatest quantity of cotton in Benin, supplying approximately 40 % of the national output (Gounou 2009). Endosulfan represents 75 % of the total quantity of insecticides applied in this region (Agbohessi et al. 2011), primarily in the form of Thionex 350 EC (51 %) and Cotofan 350 EC (24 %). Currently, Tihan represents only 1.7 % of the pesticides applied in cotton culture.

The organochlorine pesticide endosulfan is used worldwide, but it is toxic to nontarget organisms, including fish. Among the most sensitive fish are common carp (*Cyprinus carpio*) and bony bream (*Nematolosa erebi*), with median lethal concentrations (LC50) of 0.10 and 0.20 ppb, respectively (Sunderam et al. 1992). *Tilapia nilotica* is rather less sensitive (LC50 10.2–12.8 ppb) (Tellez-Banuelos et al. 2009; Werimo and Seinen 2010). During the cotton season of 2011–2012, the Beninese authorities decided to subsidize Tihan to accelerate its adoption by the cotton producers. Within the normal six applications of pesticide, two to four treatments of Tihan are recommended for protection of cotton plants, mainly against *Helicoverpa armigera* (AIC 2009). Consequently, the active components of Tihan are expected to increase in aquatic biotopes. However, there have been few studies of the toxicity of Tihan 175 O-TEQ. In

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P. T. Agbohessi (✉) · V. Gillardin · S. N. M. Mandiki ·
P. Kestemont
Research Unit in Environmental and Evolutionary Biology
(URBE), Faculty of Science, University of Namur, Rue de
Bruxelles 61, 5000 Namur, Belgium
e-mail: agbohessiprudencio@yahoo.fr

P. T. Agbohessi · I. Imorou Toko · A. Houndji
Research Unit in Aquaculture and Aquatic Ecotoxicology
(URAEAq), Department of Animal Production, Faculty of
Agronomy, University of Parakou, Parakou, Benin

an assessment of the acute effects of flubendiamide on cold- and temperate-water fish, Hall (2007) reported $LC50_{96h}$ values >60.0 ppb for rainbow trout *Oncorhynchus mykiss*, >66.5 ppb for fathead minnow *Pimephales promelas*, >67.7 ppb for bluegill sunfish *Lepomis macrochirus*, and >84.7 ppb for common carp. Reported values for the acute toxicity of spirotetramat ($LC50_{96h}$) were 2.2 ppm for bluegill sunfish, 2.54 ppm for rainbow trout, and 2.59 ppm for *Cyprinodon carpio* [Projet de Décision D'homologation du Spirotétramate (PRD) 2008]. However, we are unaware of any published studies of the effects of flubendiamide and spirotetramat on tropical fish. The present study compares the acute effects of Tihan and its active compounds on the African sharptooth catfish *Clarias gariepinus* with the effects of endosulfan (Thionex 350 EC). The African catfish is an economically important species in the Benin cotton basin (Lalèye et al. 2004). We focused on two periods of development in this fish: embryonic to larval and juvenile stages. These stages are believed to be the most vulnerable to pollution (Westernhagen 1988) because they are present during the flood, a period of intensive use of pesticides in cotton culture, and, consequently, of high concentrations in the aquatic environment.

Materials and Methods

Chemicals

Tihan 175 O-TEQ and Thionex 350 EC were purchased from the Société de Distribution des Intrants (Benin). The formulations and chemical properties of the active ingredients are listed in Table 1. Tihan is a milky-white liquid, and Thionex is a light yellow-coloured liquid. Both were stored at ambient temperature. Flubendiamide (CAS no. 272451-65-7) and spirotetramat (CAS no. 203313-25-1) were obtained from Sigma-Aldrich Chemicals (Steinheim, Germany) as powders with >98 % purity. Water used in the preparation of test solutions was tested for quality (nitrate 21.11 ± 0.03, nitrite 0.03 ± 0.01 mg/L, and total hardness 83.0 ± 0.3 mg/L). Tihan, Thionex, and spirotetramat stock solutions were dissolved in water without a carrier solvent. Because of its low solubility in water (0.0299 mg/L), flubendiamide was first dissolved in acetone (solubility in acetone is 102 g/L). All working stock solutions were made immediately before the tests.

Tests on Embryo-Larval Stages

Biological Material

Artificially fertilized eggs were obtained from African catfish reared in recirculating water at a local farm (Royal Fish Benin). Full siblings from one male (632.1 ± 7.1 g) and one female (418.5 ± 9.6 g) fish were used for the biotests. The parents were individually acclimated for 12 days in plastic tanks (120 L) at the Unité de Recherche en Aquaculture et Écotoxicologie Aquatique, University of Parakou, Benin, according to Organisation for Economic Co-operation and Development (OECD) guideline 203. They were fed twice daily with at 2 % of their biomass (Hogendoorn 1983) with a commercial food for catfish (6-mm pellets, 40 % crude protein; Coppens, Netherlands).

Experimental Design and Handling

The experimental design incorporated 18 tanks each for the Tihan, Thionex, and spirotetramat treatments (five concentrations and a zero-concentration control in triplicate) and 21 tanks (five concentrations, a control, and a solvent control in triplicate) for flubendiamide. Each tank (5 L) was equipped with an air diffuser, which ensured full oxygenation of water. Approximately 200 mg fertilized eggs (~100 eggs) were incubated in a trough placed in each aquarium filled with 3.75 L test solution. The eggs were completely submerged and spread out so they did not touch each other. Exposures to the pesticides were made under static conditions to avoid disturbing them during incubation in accordance with OECD guidelines 203 and 210 (OECD 1992a, b) with some modifications. During the tests, the photoperiod was maintained at 12 h light to dark. The acute toxicity procedure was preceded by 48-h range-finding tests to determine the concentrations at which the pesticides were lethal to eggs (data not shown). These preliminary tests, which included the period from egg fertilization to larval hatching, were performed at nominal concentrations of 0, 1, 10, 100, 500, and 1000 ppm for Tihan and Thionex; 0, 0 + acetone, 1, 10, 100, 500, and 1000 ppm for flubendiamide; and 0, 0.1, 1, 10, 50, and 100 ppm for spirotetramat. The nominal concentrations in the definitive bioassays were as follows: Tihan: 0 (control), 7.5, 15, 30, 60, and 120 ppm; Thionex: 0.0 (control), 5, 25, 75, 100, 150, and 285 ppm; flubendiamide: 0 (control), 0 + acetone (solvent control), 0.25, 0.5, 1, 2, and 3 ppm; and spirotetramat: 0 (control), 2.5, 5, 10, 20, and 30 ppm. The solvent control contained the same concentration of acetone (29.4 ppm) present in all flubendiamide treatments. Controls and treatments were run simultaneously.

Nominal concentrations were not confirmed by chemical analyses. Precise measurement of the actual concentrations was considered to be of minor importance in these series of increasing concentrations. In addition, the half-lives of flubendiamide (20.2 days [Australian Pesticides and Veterinary Medicines Authority (APVMA) (2009)] and spirotetramat (8.6–47.6 days; PRD 2008; U.S. EPA 2008) in neutral and acidic environments are greater than the

Table 1 Properties of the pesticides tested for acute toxicity

Trade name	Formulation	Active ingredient and concentration	Water solubility (mg/L)	Log K_{ow} t pH 7	Vapor pressure (Pa)	DT50 in water (days)	Reference
Tihan	175 O-TEQ (oil toxicity equivalence)	Flubendiamide (100 g/L) (phthalic acid diamide)	0.0299 ± 000287 (pH 4–10)	4.14	$<10^{-4}$	20.2	Tohnishi et al. (2005), Sattelle et al. (2008), APVMA (2009)
		Spirotetramat (75 g/L) (tetramic acid derivative)	29.9 (pH = 7)	2.51	5.6×10^{-9}	8.6–47.6	PRD (2008), U.S. EPA (2008)
Thionex	350 EC (emulsifiable concentrate)	Endosulfan (350 g/L) (organochlorine cyclodienes)	$\alpha = 0.32$ $\beta = 0.33$ at 20 °C	3.55	83×10^{-5}	4–7	Mackay et al. (1997), Tomlin (2000)

water-renewal times in our experiments. Furthermore, the active components are not very volatile (vapor pressures: flubendiamide $<10^{-4}$ Pa; spirotetramat 5.6×10^{-9} Pa). Endosulfan is stable at ambient temperature (CCME 2010). Although its vapor pressure (0.83 mPa at 20 °C) indicates that it is semi-volatile (Tomlin 2000), its half-life of 4–7 days in natural water is greater than the water-renewal time in our experiments. We therefore did not expect a significant quantity of these compounds to be lost by volatilization during the study.

Water temperatures (26.8 ± 0.9 °C), pH (7.3 ± 0.3), and dissolved oxygen concentrations (5.7 ± 0.8 mg/L) were measured daily in each aquarium. The number of fertilized eggs in each trough was counted at the beginning of the incubation. At 4-h intervals, the proportions of hatched larvae, dead eggs, and eggs/larvae with abnormalities (*e.g.*, loss of swimming coordination, dead embryo within the egg, nonmotile larvae, and larvae with curved or short tails) were recorded. The hatching rate was calculated as the percentage of fertilized eggs from which larvae hatched. Unhatched eggs that had not decayed were immediately observed under a microscope for determination of the percentage of dead embryos within the eggs. At hatching, larvae with tail deformities were classified as curved or short-tailed larvae. The permeability factor (PF) of the chorion to the pesticide was considered in the interpretation of differences in toxicity of pesticides to the eggs and embryos [$\mathrm{PF} = 11.1 \times \log K_{ow} + 3.97$, where K_{ow} is the *n*-octanol–water partition coefficient of the pesticide (Helmstetter and Alden 1995)].

Tests on Juvenile Fish

Biological Material

Juvenile African catfish (3.0 ± 0.8 g) were purchased from Royal Fish Benin, where they were produced artificially from parents reared in recirculating water conditions. Fish were acclimated for 12 days in 60-L aquaria at a stocking density of one fish per liter according to OECD guideline 203. They were fed twice daily at 4 % of their biomass until 24 h before the beginning of the test with a commercial dry feed (2-mm pellets, 45 % crude protein; Coppens).

Experimental Design and Handling

The acute test for juvenile fish was performed according to OECD guidelines 203 (1992b) with some modifications. As for the egg-to-larval stages, 18 or 21 aquaria (30 L) were used for each pesticide, including three replicates/treatment. Fish were not fed during the experiment. A few minutes after the preparation of the test solutions, ten juvenile fish were carefully placed into each tank. Initial range-finding tests were performed to ascertain the concentration range to be used in the definitive tests. The nominal concentrations were as follows: 0, 1, 10, 50, 100, and 500 ppm for Tihan; 0, 0.25, 0.5, 1, 2, and 5 ppm for Thionex; 0, 0 + acetone, 40, 80, 160, 320, and 640 ppm for flubendiamide; and 0, 0.5, 1, 10, 50, and 100 ppm for spirotetramat (data not shown). In the definitive tests the nominal concentrations were as follows: Tihan: 0 (control), 1, 5, 10, 15, 20, and 25 ppm; Thionex: 0 (control), 0.1, 0.2, 0.3, 0.4, and 0.5 ppm; flubendiamide: 0 (control), 0 + acetone (solvent control), 1, 3, 5, 10, and 20 ppm; and spirotetramat: 0.0 (control), 0.5, 1, 2.5, 5, 10, and 20 ppm. The solvent control contained the same concentration of acetone (196 ppm) present in all flubendiamide treatments. The tests on juvenile fish were performed in semi-static systems with daily renewal of 75 % of the aquarium water. Water-quality parameters were measured daily in all aquaria using standard methods (temperature 27.0 ± 0.6 °C, pH 7.0 ± 0.3, and dissolved oxygen 5.6 ± 0.7 mg/L). Signs of stress, such as loss of coordination, unusual lethargy, erratic behavior, or gasping for air, were monitored throughout the experimental period. The cumulative mortality of fish was recorded at 24-, 48-, 72-, and 96-h intervals.

Data Analysis

Egg and juvenile fish survival values were transformed into logits, and corresponding test concentrations were converted to logarithms. The 48-h LC50 ($LC50_{48h}$) values were estimated for embryo-larval stages, and 96-h LC50 ($LC50_{96h}$) values were estimated for juvenile fish. The no–observed effect concentration (NOEC) and the lowest–observed effect concentration (LOEC) were determined by comparison with the control treatments using Dunnett's test. Normality of data was first assessed using Shapiro–Wilk's test. All values were determined using the computer program ToxCalc v5.0.32 (ToxCalc 2008; Mckinleyville, CA, USA).The incidence rates of other parameters (hatching rates, loss of swimming coordination of larvae, dead embryos, nonmotile larvae, curved or short-tailed larvae) were analyzed by one-way analysis of variance analysis (ANOVA) I. Means were compared with control values by Dunnett's test with $p < 0.05$ being considered statistically significant.

Results

Acute Toxicity of Pesticides to Embryo-Larval Stages

Egg/Embryo Mortality

Pesticides with greater PF values had lower LC50, NOEC, and LOEC values (Fig. 1). Thus, Tihan, which had high PF, had lower LC50, NOEC, and LOEC values than Thionex, for which PF was low. Similarly, flubendiamide, which had high PF, showed lower LC50, NOEC, and LOEC values than spirotetramat, for which PF was low.

Hatching Rate

The effect of increasing concentrations of each pesticide or active compound on hatching rates is shown in Fig. 2. The rates in controls varied from 63 to 70 % and were significantly greater than in all treatments except for flubendiamide at the lowest concentration (0.25 ppm). Hatching rate decreased with increasing concentrations of all four agents tested. Moreover, all embryos died without hatching at the highest concentrations tested; i.e., 60 and 120 ppm Tihan, 285 ppm Thionex, 0.5–3 ppm flubendiamide, and 20–30 ppm spirotetramat (Fig. 2a through 2d).

Deformity Rate

The types of larval deformities differed among the pesticides and compounds (Table 2). Apart from an increase in embryo mortality at high concentrations, Tihan did not induce marked larval deformity at hatching. Loss of swimming coordination was induced by spirotetramat at low concentration (2.5 ppm). High rates of nonmotile larvae and larvae with curved or short tails at hatching were observed for flubendiamide. Thionex induced a lower rate of deformity but a greater rate of nonmotility than did flubendiamide. No marked deformities were observed in pesticides tested at their respective LOEC concentration, except for spirotetramat, in which loss of swimming coordination was observed at its LOEC concentration (2.5 ppm).

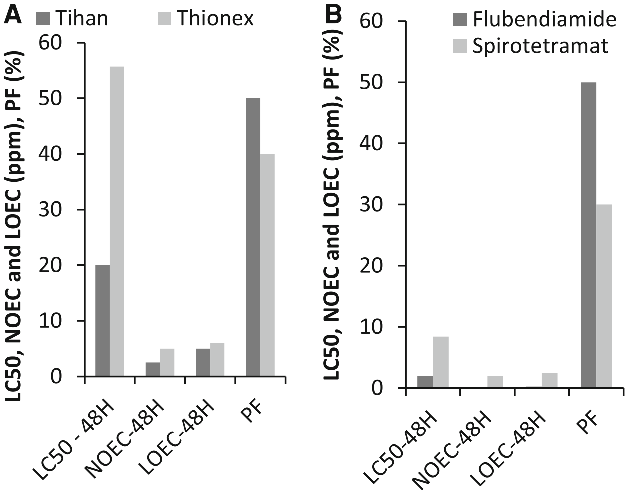

Fig. 1 Comparison of the 48-h acute toxicities of Tihan and Thionex (**a**) and of flubendiamide and spirotetramat (**b**) LC50, median lethal concentration; NOEC, no effect concentration; LOEC, lowest observable effect concentration; PF, permeability factor

Acute Toxicity of Pesticides to Early Juvenile Fish

The 96-h cumulative mortality of juvenile African catfish (Fig. 3a) indicated that Thionex was more toxic to this fish than was Tihan. Tihan was less toxic than were its active compounds (Fig. 3b). This pattern was also reflected in the high calculated $LC50_{96h}$ values (Table 3) for Tihan and its active compounds, flubendiamide and spirotetramat (8.79, 4.73, and 6.01 ppm, respectively), compared with Thionex (0.22 ppm). This latter value of Thionex corresponds to 0.077 ppm endosulfan as active compound. Fish displayed similar disruption of their swimming behavior (upside-down swimming, twisting and turning movements) before dying in 10 ppm Tihan and in 5 ppm Tihan active compounds.

Interestingly, fish that died in Tihan and its active compounds had their eyes closed, but it was noted that the eyes were wide open after Thionex treatment. Thionex caused erratic swimming, violent movements, and disorientation. Before death, movements were uncoordinated, activity was decreased, and fish vertically were orientated. Others fish were observed to exhibit curvature of the spine

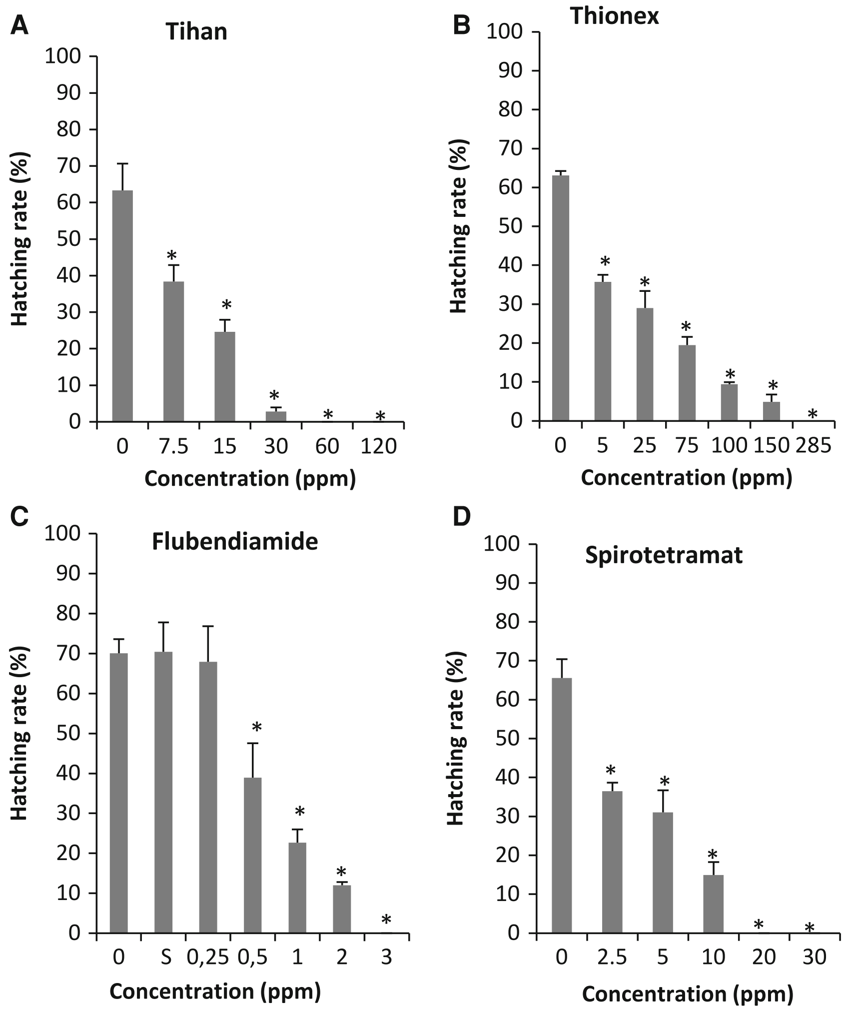

Fig. 2 Effect of increasing the acute doses of agricultural pesticides on the hatching rate of eggs of *C. gariepinus*. Values are mean ± SD ($n = 3$). *Asterisk* significantly different from the control treatments ($p < 0.05$, Dunnett's test). *S* solvent control

Table 2 Rates of deformity caused by agricultural pesticides

Tihan (ppm)	0	7.5	15	30	60	120	
Loss of swimming coordination (%)	3.20 ± 2.76	9.02 ± 5.80	12.18 ± 7.63	–	–	–	
Dead embryos in the egg (%)	0	0.29 ± 0.51	0.97 ± 1.68	10.5 ± 5.07*	–	–	
Spirotetramat (ppm)	0	2.5	5	10	20	30	
Loss of swimming coordination (%)	1.37 ± 0.35	10.10 ± 3.64*	13.54 ± 7.94*	37.66 ± 5.49*	–	–	
Flubendiamide (ppm)	0	0 acetone	0.25	0.5	1	2	3
Immobility (%)	0	0	1.95 ± 1.72	7.27 ± 1.60*	10.35 ± 1.27*	19.56 ± 9.95*	–
Larvae with curved or short tails (%)	1.71 ± 0.96	1.69 ± 1.48	2.70 ± 2.48	3.33 ± 2.77	4.16 ± 0.14*	5.56 ± 3.62*	–
Thionex (ppm)	0	5	25	75	100	150	285
Immobility (%)	0	0	5.2 ± 4.44	12.55 ± 10.93	28.33 ± 3.14*	–	–

Mean ± SD ($n = 3$)

* Significantly different from the corresponding control treatment (Dunnett's test, $p < 0.05$)

and vertical movements apparently associated with loss of equilibrium. All of these features were more pronounced with increasing concentrations of the toxicant. Consequently, the percentage of survivors decreased with increasing concentrations of the toxicants in water. We also observed skin lesions in fish at high concentrations of Thionex.

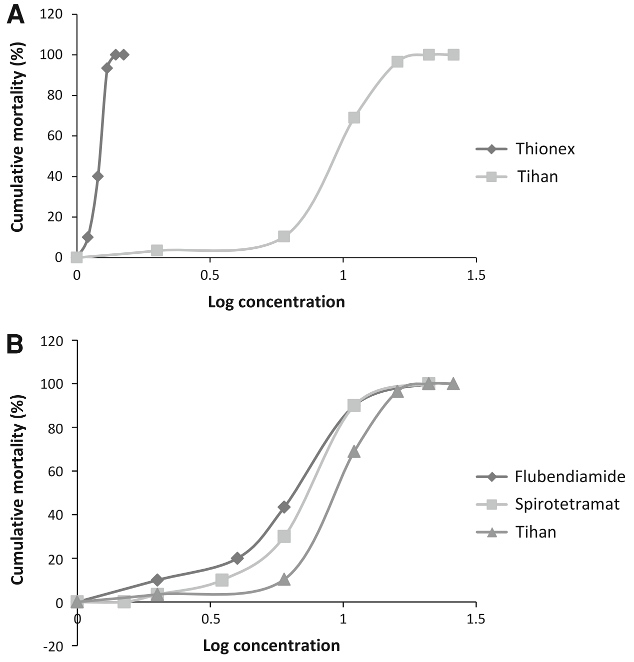

Fig. 3 Cumulative 96-h mortalities of juvenile *C. gariepinus* versus pesticide concentration. **a** Comparison of Tihan and Thionex. **b** Comparison of Tihan and its active ingredients, flubendiamide and spirotetramat

Table 3 $LC50_{96h}$, NOEC, and LOEC values for African catfish treated with different pesticides or active compounds

Pesticide or active compound	LC50 (ppm)	LC50 95 % CI (ppm)	NOEC (ppm)	LOEC (ppm)
Tihan	8.79	7.99–9.43	3	6
Flubendiamide	4.73	3.41–6.40	1	3
Spirotetramat	6.01	5.53–6.51	2.5	5
Thionex	0.22	0.19–0.24	0.05	0.1

Discussion

Effects at the Embryo-Larval Stage

After fertilization and water immersion, eggs absorb large quantities of water by osmosis. The chorion becomes distended and is then hardened by the action of enzymes. It separates from the yolk sac to form the perivitelline space. Before this hardening process, the fish chorion is permeable to water and small molecules (*e.g.*, O_2). According to Helmstetter and Alden (1995), the chorion is also permeable to lipophilic molecules, including pollutants with high n-octanol-water partition coefficients (K_{ow}). Pollutants with high log K_{ow} have high PF. Pollutants with high PF more readily penetrate the chorion than those with low PF and have been observed to cause a precipitation of egg proteins (OECD 1992a).

Accordingly, we found that the toxicity of pollutants to African catfish eggs was related to their K_{ow}. Tihan ($LC50_{48h}$ 20 ppm) was more toxic to *C. gariepinus* eggs than was Thionex ($LC50_{48h}$ 55.68 ppm), and the toxicity of flubendiamide ($LC50_{48h}$ 2 ppm) was greater than that of spirotetramat ($LC50_{48h}$ 8.44 ppm). Similar findings were reported by Nguyen et al. (1999), who showed that sodium pentachlorophenate (NaPCP; PF = 70 %) was more toxic than malathion ($p = 30$ %) to eggs of African catfish. In our study, Tihan was less toxic to *C. gariepinus* eggs than were its active components (flubendiamide and spirotetramat). This might be explained by the effects of adjuvants or additives that alter the structure or chemical properties of the active component of Tihan. Other factors that might influence PF of the chorion include the compound polarity, steric factors, functional groups, and structural configuration (Helmstetter and Alden 1995).

In the control groups, hatching rates ranged from 63 to 70 %, values comparable with those obtained by Haylor and Mollard (1995) (66–82 %) and Rukera Tabaro et al. (2005) (67 %) but lower than those reported by Mahmoud et al. (2009) (95 %) for eggs incubated in Zug bottles. Hatching is a critical period of embryogenesis that depends on biochemical and behavioral processes, including digestion of the chorion by hatching gland enzymes and movements of the embryo to open the chorion. Pollutant exposure might delay or prevent hatching by affecting both of these processes (De Gaspar et al. 1999; Hagenaars et al. 2011). Each of the tested pesticides and active compounds decreased the hatching rate in a concentration-dependent manner. For Tihan, Thionex, flubendiamide, and spirotetramat, inhibition of hatching was greater at concentrations >7.5, >5, >0.5, and >2.5 ppm, respectively, and was not specific to the compound tested. Similarly, Nguyen et al. (1999) showed that concentrations of NaPCP >1 ppm delayed embryo development of *C. gariepinus*. Köprücü and Aydin (2004) found that concentrations >0.005 ppb of deltamethrin decreased the hatching success of common carp. Hagenaars et al. (2011) also found that perfluorooctanoic acid at concentrations >1 ppm caused a decreased hatching of zebrafish *Danio rerio*, and Li et al. (2011) found that 1-bromo-3-chloro-5,5-dimethylhydantoin at concentrations >4 ppm caused decreased hatching rate in zebrafish.

Penetration of Tihan into the eggs possibly led to energy depletion to levels insufficient to support escape from the eggshell (Varo et al. 2006). This could explain the observation of dead embryos within the eggshell, particularly at 30 ppm Tihan. Köprücü and Aydin (2004) also observed the death of embryos in eggs of common carp at concentrations of deltamethrin >0.005 ppb. Thionex and flubendiamide might also have caused energy depletion, albeit to a lesser extent, thus explaining the observation of nonmotile newly hatched larvae at concentrations >100 and >0.5 ppm, respectively. Beyger et al. (2012) observed nonmotile larvae of Florida flagfish *Jordanella floridae* after exposure to 10 ppb endosulfan for 96 h. Loss of swimming coordination in larvae was observed in the Tihan-treated groups, but this was much more marked in spirotetramat treatment, even at the lowest concentration of 2.5 ppm. This behavior was observed by Jin et al. (2009) in zebrafish at 50–200 ppb bifenthrin and by Beyger et al. (2012) in Florida flagfish at concentrations of 10 ppb endosulfan. Curved or short-tailed larvae were particularly noted in flubendiamide-treated groups. Effects of perfluorinated compounds on the tail shape of zebrafish larvae were reported by Hagenaars et al. (2011). The probable causes of the tail deformities were apoptosis in the tail area, decreased cardiac output, and changes in the muscle fibers of the tail (Hagenaars et al. 2011). The latter explanation seems more plausible for flubendiamide because its mode of action in insects is to target the ryanodine receptor, an output channel for calcium ions (Ca^{2+}) involved in muscle contraction (Ebbinghaus-Kintscher et al. 2006; Masaki et al. 2006). Several studies—such as those of Jin et al. (2009), Li et al. (2011), and Beyger *et al.* (2012) cited previously—have also reported axial developmental malformations in fish larvae affected by pollutants.

Effects at the Juvenile Stage

Flubendiamide $NOEC_{96h}$ (1 ppm), $LOEC_{96h}$ (3 ppm), and $LC50_{96h}$ (4.73 ppm) for juvenile *C. gariepinus* were greater than those obtained during the evaluation of this compound by Hall (2007), who reported $LC50_{96h}$ values >60 ppb in rainbow trout, >67.7 ppb in bluegill sunfish, >66.5 ppb in fathead minnow, and >84.7 ppb in common carp. However, in this latter study, the acute tests were performed under static conditions. Under these conditions, flubendiamide may be partially transformed into its derivatives (des-iodo-flubendiamide, 3-hydroxyflubendiamide, and 3-hydroxy-perfluoroalkyl-flubendiamide), which are more toxic (Tohnishi et al. 2005). In addition, these were cold- and temperate-water species, which may not be comparable with tropical species, such as the African sharptooth catfish.

Spirotetramat was less toxic than flubendiamide ($NOEC_{96h}$ 2.5 ppm, $LOEC_{96h}$ 5.0 ppm, $LC50_{96h}$ 6.01 ppm). This $LC50_{96h}$ value is greater than that obtained under static conditions for bluegill sunfish (2.2 ppm), rainbow trout (2.54 ppm), and *C. carpio* (2.59 ppm) (PRD 2008). Here too, under static conditions, spirotetramat may be partially transformed into derivatives, such as spirotetramat-enol and ceto-hydroxy-spirotetramat, both of which are more toxic (Bruck et al. 2009). Species-specific differences might also explain variations in sensitivity among *C. gariepinus* and other species. LC50 values found in our survey were much lower than reported for the cladoceran *Ceriodaphnia dubia* ($LC50_{48h}$ 23.8 ppm) (Chen and Stark 2010).

Tihan was less toxic to juvenile fish ($NOEC_{96h}$ 3 ppm, $LOEC_{96h}$ 6 ppm, $LC50_{96h}$ 8.79 ppm) than were its active ingredients, flubendiamide and spirotetramat. There is little published information on the acute effects of Tihan on fish. The previously mentioned $LC50_{96h}$ value is greater than values reported for *C. gariepinus* for lindane [0.38–1.29 ppm (Omitoyin et al. 2006; Lawson et al. 2011)], for glyphosate [0.295 ppm (Ayoola 2008)], and for dieldrin [11.7 ppb (Lamai et al. 1999)]. It is similar to $LC50_{96h}$ values for this fish for malathion [8.22 ppm (Zubair 2012)] but less than for diazinon [11.80 ppm (Nwani et al. 2012)]. In marked contrast to control fish, we observed upside-down swimming and turning behavior in juvenile African catfish before death at concentrations >5 ppm flubendiamide and spirotetramat and >10 ppm Tihan. These behaviors appear to be specific to Tihan and its components.

The $LC50_{96h}$ values estimated for Thionex (endosulfan) for juvenile *C. gariepinus* indicated that Thionex was more toxic than Tihan, flubendiamide, and spirotetramat. The value for endosulfan (0.077 ppm) was comparable with that found by Yekeen and Fawole (2011) for the same species and development stage (0.052 ppm), but it was much lower than values reported for *Heteroneustes fossilis* [4.7–5.0 ppm (Singh and Narain 1982)] or *Channa punctatus* [5.78 ppm (Khillare and Wagh 1987)]. However, it was much greater than the $LC50_{96h}$ values for endosulfan reported for common carp [0.1 ppb (Sunderam et al. 1992)], *Anguilla anguilla* [33.7 ppb (CCME 2010)], *T. nilotica* [10.2–12.8 ppb (Tellez-Banuelos et al. 2009; Werimo and Seinen 2010)], *T. mossambicus* [3.6 ppb (Kumar et al. 2011)], and juvenile *C. gariepinus* [0.77 ppb (Ezemonye and Ikpesu 2011)]. The large difference in the value of $LC50_{96h}$ between our study and that of Ezemonye and Ikpesu (2011) could be explained by the adjuvants contained in Thionex (637 g/L hydrocarbon liquid), which would decrease the toxicity of endosulfan. The mortality pattern observed in juvenile fish treated with Thionex in our study may be attributed to skin lesions caused by exposure to this pesticide, which has been reported to weaken disease resistance and the immune response of organisms (Ezemonye and Ikpesu 2011; Yekeen and Fawole 2011).

Erratic swimming, violent movements, physical disorientation, and loss of equilibrium have previously been reported in *C. gariepinus* exposed to endosulfan (Ezemonye and Ikpesu 2011; Yekeen and Fawole 2011). Bhatia et al. (2004) reported similar behavioral changes to those observed here in *H. fossilis* treated with this compound. The Asian swamp eel (*Monopterus albus*) also exhibited these behaviors when exposed to concentrations of 0.01–10 ppb endosulfan for 96 h (Siang et al. 2007). The abnormal behavior observed in *C. gariepinus* in response to endosulfan may be explained by its known effect on the central nervous system. Endosulfan binds to the picrotoxin site in the GABA complex and impairs the normal function of the GABA channel (chloride flux is inhibited), thus leading to hyperexcitation (Coats 1990; Harris et al. 2000).

Conclusion

Tihan, a pesticide now largely used in Benin to replace endosulfan, was more toxic to African catfish eggs than the endosulfan-based formulation Thionex. The active components of Tihan were even more toxic to eggs, with flubendiamide being more toxic than spirotetramat. In contrast, for juvenile fish, Thionex was much more toxic than Tihan, flubendiamide, or spirotetramat. Tihan, spirotetramat, and Thionex were more toxic to juvenile fish than to eggs, but flubendiamide was more toxic to eggs than to juvenile fish. These observations question whether Tihan is really less damaging to the environment than endosulfan. Further studies should be directed toward the chronic effects of these compounds on exposed fish.

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