



Investigation of acute toxicity of permethrin on guppies *Poecilia reticulata*

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Abstract

Permethrin, a synthetic pyrethroid pesticide and potential toxic pollutant contaminating aquatic ecosystems, was investigated in the present study for acute toxicity. Guppy fish (*Poecilia reticulata*) were selected for the bioassay experiments. The experiments were repeated 3 times and the 48-h LC₅₀ was determined for the guppies. The static test method of acute toxicity test was used. Water temperature was regulated at 20 ± 1 °C. In addition, behavioral changes at each permethrin concentration were observed for the individual fish. Data obtained from the permethrin acute toxicity tests were evaluated using the probit analysis statistical method. The 48-h LC₅₀ value for guppy was estimated as 245.7 µg/l. Values in the range of 0.05–97.0 µg/l have been reported for various other fish species.

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1. Introduction

Synthetic analogs of the pyrethrins, extracts from the ornamental *Chrysanthemum cinerariaefolium*, have been developed to circumvent the rapid photodegradation encountered with the insecticidal natural pyrethrins. The pyrethroids are widely used in field pest control (including mosquito control programs: Kaneko et al., 2000), as household pesticides, and as veterinary and human pediculicides, and are among the most potent insecticides known (Smith and Stratton, 1986). The widespread use of these pesticides consequently leads to the exposure of manufacturing workers, field applicators, the ecosystem and finally the public to the possible toxic effects of these pesticides.

Many products containing permethrin are classified as “Restricted Use Pesticides” by the US EPA because of permethrin’s toxicity to fish. Permethrin is classified as a toxicity class II (moderately toxic) or III (slightly toxic) chemical, depending on the formulation (<http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs>).

Pyrethroids have been reported to be extremely toxic to fish, some beneficial aquatic arthropods (for example, lobster and shrimp); and to honey-bees in laboratory tests. The 48-h LC₅₀ of permethrin for rainbow trout was 5.4 µg/l and in bluegill sunfish and salmon, 1.8 µg/l. It is extremely toxic to bees. Severe losses may be expected if bees are present at treatment time, or within a day after treatment (Bradbury and Coats, 1989; <http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs>). Concerning fish toxicity, emulsifiable concentrate preparations of permethrin enhanced its toxicity twofold. Neurotoxin binding properties differ in fish and mammals leading to low mammalian toxicity.

Lethality varies inversely with water temperature, particularly between 10 and 20 °C, and with body weight between 1 and 50 g. There was a 10-fold difference

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between the 96-h LC₅₀ values at 10 and 20 °C. At 15 °C, a large trout (200 g) was considerably more (about 100 times) tolerant than a small fish (1 g) (WHO, 1990). Table 1 depicts acute toxicity of permethrin to selected fish species as published in the literature (key sources are as reviewed by Bradbury and Coats, 1989; WHO, 1990; and <http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs>; others are directly from the open literature). The Columbia Environmental Research Center Acute Toxicity Database reports permethrin toxicity as an average of 7–8 µg/l to bluegill; brook trout and rainbow trout are reported more sensitive than bluegill (<http://www.cerc.cr.usgs.gov/data/acute/acute.html>).

Pyrethroid toxicology in mammals (including man), birds, amphibians and both terrestrial and aquatic invertebrates has also been reviewed (Bradbury and Coats, 1989; WHO, 1990; Siegfried, 1993). Toxicity is highly dependent on stereochemical structure. Most products however, are mixtures of isomers. Toxicity of permethrin is dependent on the ratio of the isomers present; the *cis*-isomer being more toxic. Several mosquito larvicides and adulticides including resmethrin and permethrin were evaluated for toxicity to standard test (in-house cultures) and resident organisms to measure effects of mosquito control pesticides to aquatic fauna with special emphasis to non-target organisms (Yameogo et al., 1992, 1993; Milam et al., 2000; Crosa et al., 2001; Yameogo et al., 2001).

Pyrethroids are especially advantageous for use in northern climate zones, since they exhibit a negative temperature coefficient of toxicity. They are also considered to be relatively non-persistent, therefore are not expected to biomagnify through the food chain. Maximum bioconcentration factors ranged from 698X for whole fish (deltamethrin) to 6090X (bifenthrin); permethrin's bioaccumulation in fish were: 570–610X for whole fish, 950–1000X in the viscera, 180–230X in the fillet (<http://www.epa.gov/oscpmont/sap/1999/february/pyreth.pdf>). Toxic effects of pyrethroids on non-target organisms have been reviewed and reported to be in the ppb toxicity range (Smith and Stratton, 1986). In both the laboratory and field, adsorption to soil of pyrethroids substantially reduces toxicity. Therefore in the field most of the affected organisms showed rapid recovery. The environmental fate and effects of synthetic pyrethroid insecticides have been summarized (Hill, 1989).

Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of fish to aqueous pyrethroid exposures. Fish seem to be deficient in the enzyme system that hydrolyzes pyrethroids. Metabolism in fish is largely oxidative (Demoute, 1989). Fish make intimate contact with the surrounding water through the gills. The potential hazard to fish is due to its heavy use in many aquatic mosquito larvicidal programs. Synergistic interactions between the active ingredient and

other components of the formulation should be taken into consideration when evaluating toxicity.

This study was conducted to determine the acute toxicity of permethrin, to the guppy (*Poecilia reticulata*; recommended for acute toxicity testing by OECD and APHA, AWWA, WEF) using the static test system.

2. Materials and methods

Male, adult guppies were obtained from a local breeder in Ankara and brought to the laboratory within 30 min in plastic bags with sufficient air. The plastic bags were placed into the maintenance aquarium for about 30–35 min for acclimatization. Then the bags were cut open and the fish were allowed to swim into the aquarium water. Test chambers were glass aquaria of about 25 l capacity. Temperature was regulated at 20 ± 1 °C by using heaters. At the time of dosing air was turned off; it was on at all times otherwise. The water was continuously aerated for several days before putting the fish in, to remove chlorine.

Test chambers were filled with 20 l of tap water. Characteristics of this aquarium water were as follows: temperature 20 ± 1 °C, dissolved oxygen 7.8–8.0 mg/l and conductivity 0.212–0.260 mS.

Following the preliminary experiment, all determinations were repeated three times. Groups of experimental animals, each consisting of 10 individuals, were selected at random and placed into aerated aquaria. After 48 h of adaptation, the different concentrations of permethrin in acetone were added to the experimental aquaria. During the last 24 h of adaptation, and throughout the duration of the experiment, animals were not fed. Mortality was assessed at 24, 48, 72 and 96 h after the start of the tests. Dead individuals were removed immediately. Behavioral changes were followed closely.

Technical grade (94.93%) permethrin was from the Insecticide Testing Laboratory of Hacettepe University, Ankara (source: Institute of Organic Industrial Chemistry, Warsaw, Poland). Technical permethrin was stored at +4 °C until stock solution preparation. Stock solution was prepared first by warming up the permethrin to ensure efficient mixing of the isomers and then by weighing a certain amount after cooling to room temperature and diluting it in acetone to give the stock material. Dosing solutions were prepared from this stock by diluting with acetone to give the dosing concentrations of 60.0, 105.0, 157.5, 210.0, 315.0 and 525.0 µg/l. The dosing volume never exceeded 0.2 ml. Control group received acetone at the maximum acetone volume used in the dilution of the dosing concentrations. The bioassay system was as described in standardized methods (OECD, 1993; APHA, AWWA, WEF, 1998) and the national regulation (Turkish Official

Table 1
Acute toxicity of permethrin to fish species^a

Species	Duration of test (h)	Toxicity (LC ₅₀)	Reference
<i>Salmo salar</i>	96	12.0 µg/l	Mc Leese et al. (1980)
	96	8.8 µg/l	Zitko et al. (1977)
<i>Salmo gairdneri</i>	96	6.43 µg/l	Kumaraguru and Besmish (1981)
	24	25.8 µg/l	Holcombe et al. (1982)
	96	7.0 µg/l	Holcombe et al. (1982)
	48	6.0 µg/l	Mulla et al. (1978)
	24	8.0 µg/l	Mulla et al. (1978)
	96	9.0 µg/l	Warting and Walker (1983)
	48	14.0 mg/l	Glickman et al. (1981)
<i>Oryzias latipes</i>	48	41.0 µg/l	Miriamoto et al. (1976)
	24	24.0 µg/l	Rice et al. (1997)
	48	11.0 µg/l	Rice et al. (1997)
	48	3–6 µg/l	Kikuchi et al. (1984)
<i>Ictalurus punctatus</i>	96	1.1 µg/l	Jolly et al. (1978)
<i>Micropterus salmoides</i>	96	8.5 µg/l	Jolly et al. (1978)
<i>Gambusia affinis</i>	96	15.0 µg/l	Jolly et al. (1978)
	48	97.0 µg/l	Mulla et al. (1978)
	48	2.7 µg/l	Milam et al. (2000)
<i>Salvelinus fontinalis</i>	96	3.2 µg/l	Mayer and Ellersieck (1986)
<i>Pimaphales promelas</i>	96	5.7 µg/l	Mayer and Ellersieck (1986)
	24	40.3 µg/l	Holcombe et al. (1982)
	96	15.6 µg/l	Holcombe et al. (1982)
	48	range: 33.9–>75 mg/l	Milam et al. (2000)
<i>Lepomis macrochirus</i>	96	5.0 µg/l	Mayer and Ellersieck (1986)
<i>Cyprinodon variegatus</i>	24	5.46 µg/l	Tietze et al. (1995)
	48	3.02 µg/l	Tietze et al. (1995)
	96	7.8 µg/l	Schimmel et al. (1983)
<i>Cyprinodon macularis</i>	48	44.0 µg/l	Mulla et al. (1978)
<i>Channa striatus</i>	24	2.0 mg/l	Sigh and Agarwal (1999)
<i>Stripped bass (adult)</i>	24	32.8 µg/l	Tietze et al. (1995)
	96	16.1 µg/l	Rebach (1999)
	24	26.7 µg/l	Rebach (1999)
<i>Stripped bass (juvenile)</i>	24	27.0 µg/l	Tietze et al. (1995)
<i>Menidia beryllina</i>	24	4.07 µg/l	Tietze et al. (1995)
	48	2.86 µg/l	Tietze et al. (1995)
	96	0.62 µg/l	Kumaraguru and Beamish (1981)
<i>Paleomonetes pugio</i>	24	0.843 µg/l	Tietze et al. (1995)
	48	0.049 µg/l	Tietze et al. (1995)
<i>Gambusia holbrooki</i>	24	6.04 µg/l	Tietze et al. (1995)
	48	4.29 µg/l	Tietze et al. (1995)
	48	26.0 µg/l	Yameogo (1991)
<i>Polymyrus isidori</i>	48	26.0 µg/l (as 20% EC)	Yameogo (1991)
	24	40.0 µg/l (as 20% EC)	Yameogo (1991)
<i>Oreochromis niloticus</i>	24	40.0 µg/l (as 20% EC)	Yameogo et al. (2001)
	48	27.0 µg/l (as 20% EC)	Yameogo et al. (2001)
<i>Tilapia zillii</i>	24	75.0 µg/l	Yameogo et al. (2001)
	48	49.0 µg/l	Yameogo et al. (2001)

^aReferences not listed in the References section have been as referred to by Bradbury and Coats (1989) and WHO (1990).

Gazette, 1991). The selected species is also as recommended in these references. LC₅₀ and 95% confidence limits were calculated by a computer program (US EPA, 1999).

3. Results

The calculated 48-h acute LC₅₀ value (95% confidence limits) of technical permethrin, dissolved in acetone, using a static bioassay system to adult, male guppies *Poecilia reticulata* was 245.7 µg/l (198.1–316.5). Control mortality was zero. The results show that permethrin is highly toxic to fish; but it is less toxic to guppies than to most other species as depicted in Table 1. Results are in Table 2 and Fig. 1.

Observations of behavioral response of guppies were conducted at 1–8, and every 12 h during the acute toxicity tests. The control group showed normal behavior during the test period. The changes in behavioral response started 1 h after dosing. The 60 µg/l (lowest) concentration had similar behavior with the control group. Observed behavioral changes/effects were typical of neurotoxin toxicity: less general activity than control group, loss of equilibrium, erratic swimming and staying motionless at a certain location generally at mid-water level for prolonged periods. Fish exposed to 105.0 µg/l showed less general activity with occasional loss of equilibrium. Loss of equilibrium become more frequent in the 157.5 µg/l concentration. The 210.0 concentration group stayed motionless close to the water surface and later fell to the aquarium bottom in an uncontrolled manner. Fish in the 315.0 µg/l concentration showed loss of equilibrium, much decreased general activity and lying motionless on the bottom on their backs.

The highest concentration of 525.0 µg/l showed all responses at high intensities: the loss of equilibrium, hanging vertically in water, rapid gill movement, erratic swimming, sudden swimming motion in a spiral fashion, after long periods of motionlessness, prolonged and

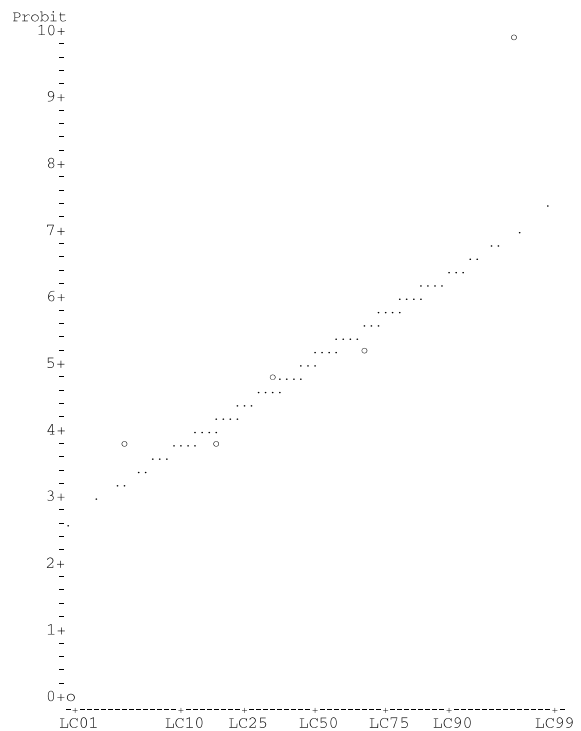


Fig. 1. Plot of adjusted probits and predicted regression line for permethrin to guppies (*Poecilia reticulata*).

motionless lying down on the aquarium bottom and suddenly starting to move. Our results are in agreement with Rice et al. (1997).

4. Discussion

The 48-h LC₅₀ value of permethrin in guppies was found to be 245.7 µg/l in the present work and therefore we report permethrin to be toxic to fish since the LC₅₀ value is in the µg/l level. The U.S.D.A. National Agricultural Pesticide Impact Assessment Program's

Table 2

Acute 48-h toxicity of technical permethrin in adult male guppies (*Poecilia reticulata*)

Point	Concentration (µg/l)	95% Confidence limits	Slope ± SE	Intercept ± SE
LC 1.00	79.94	32.70–115.46	4.77 ± 1.09	-6.40 ± 2.58
LC 5.00	111.08	58.31–147.49		
LC 10.00	132.37	78.84–169.18		
LC 15.00	149.00	96.16–186.53		
LC 50.00	245.73	198.14–316.59		
LC 85.00	405.25	314.97–696.46		
LC 90.00	456.17	345.47–853.86		
LC 95.00	543.60	394.40–1159.96		
LC 99.00	755.31	501.60–2077.28		

Note: Control group (theoretical spontaneous response rate) = 0.0000.

EXTOXNET document (<http://ace.orst.edu/cgi-bin/mfs/01/pips/permethr.htm?8#mfs>) reports permethrin acute toxicity (LC₅₀) to fish in laboratory tests, in the ppb level. Table 1 compiles the fish toxicity data of other researchers and almost all LC₅₀ are in the ppb range. Our results, LC₅₀, are also in the µg/l range but permethrin is less toxic to guppies than to most other species listed in the table. Special attention is called to the data with *Gambusia affinis* in Table 1, another member of the Poeciliidae family similar to the guppy: 48-h LC₅₀ 2.7 and 97 µg/l and 96-h 15.0 µg/l. Mittal et al. (1994) reported deltamethrin toxicity to guppy, *Poecilia reticulata* as the most toxic of the pyrethroids studied: LC₅₀ = 0.016 ppm. Another guppy toxicity study estimated the 48-h LC₅₀ value of beta-cypermethrin as 21.4 µg/l (Polat et al., 2002).

Bradbury and Coats (1989) and WHO (1990) have reviewed the toxicology of pyrethroids in mammals, birds, fish, amphibians, and invertebrates (terrestrial and aquatic). Permethrin's bioaccumulation in fish were: 570–610X for whole fish, 950–1000X in the viscera, 180–230X in the fillet (<http://www.epa.gov/oscpmont/sap/1999/february/pyreth.pdf>). Although under field conditions permethrin is considered to pose less risk due to its high adsorption to soil, these data should be considered when assessing possible/potential ecosystem risks.

Singh and Srivastava (1999) exposed freshwater fish *Channa striatus* (Bloch) to permethrin at a dose of 2.0 mg/l (24-h LC₅₀) and found significant reduction in the activity of lactate dehydrogenase and cytochrome oxidase and enhancement in succinate dehydrogenase activity in the tissues. They concluded that the mechanism of action was blocking of aerobic as well as anaerobic metabolism in the exposed fishes.

Permethrin exposure resulted in muscarinic cholinergic receptor downregulation in fathead minnow and razorback suckers brain but the concentrations required for this effect were much greater than that observed in cold water species (Jones et al., 1998). The researchers concluded: (i) inconsistency in response in the species studied; (ii) multiple mechanisms of action of permethrin possibly affecting cholinesterase activity in a less direct manner; (iii) lipophilicity of permethrin causing a generalized perturbation of the lipid membrane reflected by allosteric changes in membrane proteins such as receptor molecules.

Rebach (1999) studied the acute toxic effects of piperonyl butoxide synergized permethrin (1:1, by active ingredient) on juvenile striped bass hybrids and showed sublethal behavioral effects such as agitated/excited behavior and ataxia.

It is interesting to note that only a few studies on the acute toxicity to fish of one of the most toxic pyrethroids, namely permethrin, are available in the open literature. Some of the published reports are only with local fish species or sensitive species. The report of

permethrin acute toxicity to the recommended/standard fish species for toxicity testing, the guppy, is for the first time in this report.

5. Conclusion

Permethrin is a highly toxic synthetic pyrethroid pesticide widely used in agriculture and vector control programs. Special attention is drawn to its heavy use in mosquito control programs which necessitates in-depth sub-chronic and chronic toxicity tests to fish species and to non-target species to be undertaken. In addition, potential risk from permethrin metabolites should be investigated to get a more complete picture in terms of toxicity.

Short-, medium-, long-term biological variations related to permethrin (as larvicide) applications was studied under the Onchocerciasis Control Programme in West Africa (Crosa et al., 2001). Field assessment of the impact of permethrin on fish communities did not suggest any reduction in catch per unit effort or species richness that could be attributed to larvicide applications. Yameogo et al. (1991) using an operational dose of 0.015 mg/l/10 min reported a risk index value of 0.37. Risk index was described as the ratio between operational dose versus 24-h LC₅₀. The low toxicity of permethrin to mammals may be misleading at this point in terms of ecotoxicology and lead to extrapolation problems to aquatic species. Delistraty (2000) in the study of examining relationships among physicochemical properties and acute toxicity endpoints of 231 chemicals in rats and trout concluded that trout aquatic LC₅₀ was predicted from rat inhalation LC₅₀ with moderate success. Therefore such data are useful in ecological risk assessment but there are limitations and uncertainties. Further work with toxicity testing methods directly on fish will be very useful in assessing possible ecological risk of these pesticides. To overcome discrepancies and potential synergistic effects from the components of the pyrethroid formulations, toxicity tests with formulations must be included together with active ingredient tests. Using only the pyrethroid active ingredient in the tests is insufficient.

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References

APHA, AWWA, WEF, 1998. Standard Methods for the Examination of Water and Wastewater, Washington, D.C.

- Bradbury, S.P., Coats, J.R., 1989. Comparative toxicology of the pyrethroid insecticides. *Rev. Environ. Contam. Toxicol.* 108, 133–177.
- Crosa, G., Yameogo, L., Calamari, D., Diop, M.E., Nabe, K., Konde, F., 2001. Analysis of the effects of rotational larviciding on aquatic fauna of two Guinean rivers: the case of permethrin. *Chemosphere* 44, 501–510.
- Delistraty, D., 2000. Acute toxicity to rats and trout with a focus on inhalation and aquatic exposures. *Ecotoxicol. Environ. Saf.* 46, 225–233, doi: 10.1006/eesa.1999.1906.
- Demoute, J.P., 1989. A brief review of the environmental fate and metabolism of pyrethroids. *Pestic. Sci.* 27, 375–385.
- Hill, I.R., 1989. Aquatic organisms and pyrethroids. *Pestic. Sci.* 27, 429–465.
- Jones, S.B., King, L.B., Sappington, L.C., Dwyer, F.J., Ellersieck, M., Buckler, D.R., 1998. Effects of carbaryl, permethrin, 4-nonylphenol, and copper on muscarinic cholinergic receptors in brain of surrogate and listed fish species. *Comp. Biochem. Physiol.* 120C, 405–414.
- Kaneko, A., Taleo, G., Kalkoa, M., Yamar, S., Kobayakawa, T., Björkman, A., 2000. Malaria eradication on islands. *Lancet* 356, 1560–1564.
- Milam, C.D., Farris, J.L., Wilhide, J.D., 2000. Evaluating mosquito control pesticides for effect on target and nontarget organisms. *Arch. Environ. Contam. Toxicol.* 39, 324–328.
- Mittal, P.K., Adak, T., Sharma, V.P., 1994. Comparative toxicity of certain mosquitocidal compounds to larvivorous fish *Poecilia reticulata*. *Indian J. Malariol.* 31, 43–47.
- OECD (Organization for Economic Co-operation and Development), 1993. OECD guidelines for testing of chemicals. OECD, Paris.
- Polat, H., Erkoç, F.Ü., Viran, R., Koçak, O., 2002. Investigation of acute toxicity of beta-cypermethrin on guppies *Poecilia reticulata*. *Chemosphere* 49, 39–44.
- Rebach, S., 1999. Acute toxicity of permethrin/piperonyl butoxide on hybrid striped bass. *Bull. Environ. Contam. Toxicol.* 62, 448–454.
- Rice, P.J., Drewes, C.D., Klubertanz, T.M., Bradbury, S.P., Coats, J.R., 1997. Acute toxicity and behavioral effects of chlorpyrifos, permethrin, phenol, strychnine, and 2,4-dinitrophenol to 30-day-old Japanese medaka (*Oryzias latipes*). *Environ. Toxicol. Chem.* 16, 696–704.
- Siegfried, B.D., 1993. Comparative toxicity of pyrethroid insecticides to terrestrial and aquatic insects. *Environ. Toxicol. Chem.* 12, 1683–1689.
- Singh, A., Srivastava, V.K., 1999. Toxic effects of synthetic pyrethroid permethrin on the enzyme system of the freshwater fish *Channa striatus*. *Chemosphere* 39, 1951–1956.
- Smith, T.M., Stratton, G.W., 1986. Effects of synthetic pyrethroid insecticides on nontarget organisms. *Res. Rev.* 97, 93–119.
- Turkish Official Gazette (Resmi Gazete), 1991. Su Kirliliği ve Kontrolü Yönetmeliği Numune Alma ve Analiz Metodları Tebliği. Zehirlilik Seyreltme Faktörü (ZSF) Tayini. Tarih: 7.1.1991, Sayı: 20106.
- US EPA, 1999. LC50 software program, version 1.00. Center for Exposure Assessment Modeling (CEAM) Distribution Center.
- World Health Organization, 1990. Permethrin, Environmental Health Criteria 94, World Health Organization, Geneva.
- Yameogo, L., Tapsoba, J.-M., Calamari, D., 1991. Laboratory toxicity of potential blackfly larvicides on some African fish species in the Onchocerciasis Control Programme Area. *Ecotoxicol. Environ. Saf.* 21, 248–256.
- Yameogo, L., Elouard, J.M., Simier, M., 1992. Typology of susceptibilities of aquatic insect larvae to different larvicides in a tropical environment. *Chemosphere* 24, 2009–2020.
- Yameogo, L., Abban, E.K., Elouard, J.M., Traore, K., Calamari, D., 1993. Effects of permethrin as simuliid larvicide on non target aquatic fauna in on African river. *Ecotoxicology* 2, 157–174.
- Yameogo, L., Traore, K., Back, C., Hougard, J.-M., Calamari, D., 2001. Risk assessment of etofenprox (vectron®) on nontarget aquatic fauna compared with other pesticides used as *Simulium* larvicide in a tropical environment. *Chemosphere* 42, 965–974.