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Influence of Fishing Technique on Organochlorine Pesticide Accumulation in Fish and its Possible Human Health Risk in the Republic of Bénin

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Abstract In Bénin different techniques are used for large-scale commercial fishing, Acadjas (enclosures constructed in the river) and Whédos (holes made in the river banks). This study aimed at assessing the extent of contamination related to these fishing techniques. Fish contained residues of DDT and its metabolites, α -endosulfan, dieldrin, aldrin, endrin and lindane. Pesticide levels were similar in fish from Acadjas and Whédos, except for higher α -endosulfan levels in fish from the Whédos. Comparing pesticide intake levels through fish consumption with tolerable daily intake levels showed that in all cases risk for human health is low.

Keywords Human health risk · Organochlorine pesticides · Fish culture techniques · Human exposure

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In the Republic of Bénin, rivers and estuaries serve as an important source of income for fishermen. Fishing in the Ouémé valley is a major activity during the year and performed by all age categories of the population. Over 4,250 fishermen are full-time working in this area together with an additional 13,000 seasonal fishermen. Fishermen exploiting the Ouémé River use a diversity of techniques, such as nets, sieving nets, and sparrow nets.

In Bénin, particular fishing techniques are used to cultivate fish in the river, including Acadjas and Whédos. Acadjas are enclosures constructed with wood or branches submersed in the river, sometimes surrounded by nets (Lalèyè 2000; Lalèyè et al. 2001). They may have different forms, square, rectangular or circular, and fish are kept in the Acadjas for 6 months–1 year. Fish in the Acadjas sometimes are fed and may gain considerable weight. Whédos consist of a hole made in the river bank when the water level is low. Whédos also have irregular shapes. Fish enter the Whédos when the water level rises. After withdrawal of the water, fish are trapped in the Whédos and may be kept there for 6–7 months to be collected before the water rises again.

Acadjas and Whédos are constructed without consideration of water quality. They may, however, be exposed to waste material dumped into the river, or to run off from agricultural land. As a consequence, fish in these systems may get exposed to chemicals, especially to agricultural pesticides. Considering the difference in the way the fish are kept, it is likely that their exposure to pesticides may also be different. Differences may be related to the level of pesticide residues in water and sediment, frequency of exposure or duration of exposure.

The purpose of this study was to investigate the pesticide levels in fish collected from the same river area but using different fishing (and cultivation) techniques on the

one hand and on the other hand to compare them with fish collected from the same river. For that purpose four different fish species were collected from Acadjas, Whédos and from the Ouémé River at Lowé in Bénin. The main objective of the present study was to quantify the extent of contamination of Acadja and Whédo fish with organochlorinated pesticides. In addition, we made an attempt to assess the potential human health risk from fish consumption.

Materials and Methods

The Lowé area is situated in the lower basin of the Ouémé River between 6°22 and 7°16 N and between 2° and 1°50 E (see for a map of the area: Yehouenou A. Pazou et al. 2006). The lower valley of the Ouémé River is bordered in the East by the basin of Yema in Nigeria, in the west by the basin of Couffo, in the North by the rocky outcrops situated to the North of Bohicon (road to Zagnanado) and in the South by Cotonou Lagoon (Colombani et al. 1972). The lower valley of the Ouémé River has a sedimentary soil (marble covered by clayey sands) with an alluvial plain, and is 5 km wide at Sagon and Gangban, 10 km at Bonou and 20 km at Hozin. The valley is flooded in periods of high water.

In 2004, fishes belonging to the species *Clarias gariepinus*, *Protopterus annectens*, *Polypterus senegalensis senegalensis* and *Parachanna obscura* were caught with sparrow nets from the river and collected from Acadjas and Whédos near Lowé. Only one fish of each species was caught. The fish were washed and stored in an ice-chest at 4°C and transported to the laboratory. Muscle tissues of each fish were collected, weighted, frozen, lyophilized and stored at –20°C before analysis.

All fish samples were analysed by the certified chemical-analytical laboratory of the Hoogheemraadschap Hollands Noorderkwartier (HHNK) in Edam. All solvents and other chemicals used were of analytical grade quality (Merck, J.T. Baker) to ensure purity.

Dissected and lyophilized fish flesh (edible muscle tissue) were ground to fine powder using a ceramic pestle and mortar. Two grams of fish powder were extracted with 200 mL acetone by shaking for 10 min, after which 20 mL of a saturated sodium sulphite solution and 100 mL petroleum ether were added. After shaking for 10 min, the suspension was filtered over a paper filter and washed two times with 500 mL deionized water to which 20 mL sodium chloride solution was added. The petroleum ether fraction was subsequently dried with sodium sulphate and volume reduced evaporation in a Kuderna-Danish rotavapor at 75–80°C.

Lipid contents of the fish were obtained by weighing and drying 1 mL of the extract.

For clean-up, the extract was placed on an aluminium oxide column and eluted with petroleum ether followed by elution over a column of deactivated silica gel, also with petroleum ether. The extracts were concentrated to 2.0 mL under a gentle stream of nitrogen gas. Analysis took place by GC (Hewlett Packard 6890) with ECD, equipped with an autosampler (Hewlett Packard injector 7673) and a Chrompack 8753 CP-Sil-8 CB column. Helium was used as the carrier gas, and organochlorine pesticides were identified by comparing their retention times with those of a standard mixture of PCBs and organochlorine pesticides. For calibration purposes, PCB155, mirex and tetrachlorobenzene were used as internal standards.

The limits of detection for all pesticides including Σ DDT (pp'-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT) was 0.1 ng/g lipid, the limit of quantification was 0.3 ng/g lipid.

Quality assurance measures included rigorous contamination control procedures (strict washing and cleaning procedures), monitoring of blank levels of solvents, equipment and other materials, analysis of procedural blanks, recovery of spiked standards, monitoring of detector response and linearity, and analysis of a reference material (dried sewage sludge). For each series of 6–8 samples, one blank and a sample of the reference material were included. Recoveries of chlorinated pesticides in the reference material were between 80 % and 110 % of certified concentrations.

To assess the risk of pesticide intake by fish consumption from Acadjas and Whédos by the local population, it was assumed that local people would eat one fish per day. On the basis of that and using the pesticide concentrations measured, for each pesticide the daily intake was estimated. Next, daily intake was normalized to an average human, weighing 60 kg. The resulting intake values were compared with the tolerable daily intake (TDI) values reported by the WHO (2004). Assuming all pesticides have the same mode of action, the toxic unit approach was adopted to enable summing the risk of the different pesticides detected (Van Gestel et al. 2011). For that purpose, a toxic unit (TU) was defined as the estimated daily intake of a pesticide by fish consumption divided by its TDI. If the sum of the toxic units approach or exceed the value of 1, this would indicate a risk for the local population.

Results and Discussion

The organochlorine pesticides pp'-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT, α -endosulfan, dieldrin, aldrin and lindane were detected at varying concentrations in fish from both Whédos and Acadjas (Table 1). In general, the highest pesticide levels were found in *P. obscura* and the

lowest ones in *P. annectens*. The different pesticide levels may be attributed to how each species bioaccumulates the pesticides, but may also be related to their activity in the water (e.g. predatory behaviour) or interaction with the sediment (see e.g. Van der Oost et al. 1996; Kidd et al. 2001). For most of the species analyzed the latter is an important factor, as they are able to survive dry periods by living in mud. Nevertheless, for most pesticides, concentrations in the same species were similar for Whédos and Acadjas. The only exception was α -endosulfan, which had higher concentrations in the fish from Whédos, especially in *C. gariepinus* and also in *P. senegalensis senegalensis*. These results may be explained by intensive use of endosulfan in the area. The low levels of endosulfan in Acadja fish may further be explained by dilution of endosulfan when entering the river, while in Whédos less dilution is possible. In addition, endosulfan is a fairly persistent compound that may be present for long period in sediments with half-lives of >45 days (Laabs et al. 2007). As a consequence, exposure of the fish in Whédos probably was more likely to be chronic while in Acadjas it was more in the form of a peak or pulse exposure related to peaks in the agricultural application of the pesticide.

The ratio of DDT/DDE often is suggested as an indication of the history of the use of DDT. When ratio is high, DDT has only recently been sprayed in the area, but when the ratio is low the more persistent metabolites DDE and DDD dominate (Gitahi et al. 2002; Dem et al. 2007; Darko et al. 2008). In the fish from the Acadjas, DDT represented 22 %–40 % of the Σ DDT while in the fish from the Whédos this was slightly lower with 11 %–29 %. In the Acadja fish, DDE made up 28 %–60 % of Σ DDT while in

the fish from the Whédos this was 36 %–63 %. These data suggest that the Σ DDT residues in fish from both fishing techniques do not result from recent DDT use.

Comparing pesticide levels for fish from Whédos and Acadjas with those from the Ouémé River was only possible for *C. gariepinus* as the other fish species were not collected from the river. Results of this comparison are shown in Table 2, using data on pesticide residues in *C. gariepinus* from different areas along the Ouémé River reported by Yehouenou A. Pazou et al. (2006). Σ DDT levels in *C. gariepinus* caught in the river at Atchakpa-Béri, Bétérou, Bonou, Houédo and Lowé areas were higher than the fish from Acadjas and Whédos near Lowé. The opposite was the case for α -endosulfan, which showed somewhat higher levels in *C. gariepinus* from Acadjas and much higher levels in fish from Whédos. Σ drin and lindane levels were higher in *C. gariepinus* from Acadjas and Whédos than in the same species caught at different sites along the Ouémé river (Table 2).

Pesticides residues levels in *P. obscura* and *C. gariepinus* collected in Acadjas and in Whédos in the Ouémé River at Lowé were also compared to the same fish species collected at Nsawan and Weija near the Densu basin in Ghana. pp'-DDE levels in *C. gariepinus* from Acadjas and Whédos (values from Table 1 expressed on a lipid content basis converted to total concentrations corresponding with 2.8–3.1 $\mu\text{g}/\text{kg}$) were in the same range as concentrations measured in fish collected at Weija (8.5 $\mu\text{g}/\text{kg}$) and Nsawan (7.2 $\mu\text{g}/\text{kg}$) in Ghana (Afful et al. 2010). The same was found for pp'-DDT levels in *C. gariepinus* from Weija (0.6 $\mu\text{g}/\text{kg}$) and Nsawan (4.4 $\mu\text{g}/\text{kg}$) (Afful et al. 2010). α -Endosulfan levels, however, were 18–200 times higher in

Table 1 Pesticide residues levels in fish collected from Acadjas and Whédos along the Ouémé River in the Republic of Bénin. Pesticide levels are expressed on the basis of lipid content in (ng/g lipid)

Pesticide	Acadja				Whédo			
	<i>Clarias gariepinus</i>	<i>Protopterus annectens</i>	<i>Polypterus senegalensis senegalensis</i>	<i>Parachanna obscura</i>	<i>Clarias gariepinus</i>	<i>Protopterus annectens</i>	<i>Polypterus senegalensis senegalensis</i>	<i>Parachanna obscura</i>
op'-DDE	<1	<2	<2	<13	<4	<2	<2	<13
pp'-DDE	140	40	168	390	134	26	136	379
op'-DDD	3	25	<8	37	10	5	22	23
pp'-DDD	110	22	30	283	44	20	73	263
op'-DDT	15	9	33	23	8	7	27	23
pp'-DDT	55	44	49	452	16	14	29	68
α -endosulfan	120	24	23	158	7,926	102	678	31
Aldrin	<1	<2	<4	<13	9	6	24	<13
Dieldrin	4	10	5	57	<4	2	11	<13
Endrin	<2	<2	<4	<13	<4	<2	<2	<13
Lindane	3	<1	<4	<13	7	5	6	<13

Table 2 Comparison of pesticide residues levels in *Clarias gariepinus* collected in Acadjas and Whédos (this study) and at others locations along the Ouémé River in the Republic of Bénin taken from Yehouenou A. Pazou et al. (2006)

	Acadja	Whédo	Atchakpa-Béri	Bétérou	Bonou	Houédo	Lowé
ΣDDT	324	212	1,384	535	1,642	789	891
Σendosulfan	120	7,926	14	23	32	33	27
Dieldrin	4	9	<0.1	<0.1	<0.1	<0.1	10
Lindane	3	7	<0.1	<0.1	<0.1	<0.1	<0.1

All concentrations are given in ng/g lipid

C. gariepinus from Whédos (166 µg/kg) compared to fish from Nsawan and Weija (8.7 and 0.8 µg/kg, respectively) in the Densu basin (Afful et al. 2010). pp'-DDE, pp'-DDD and pp'-DDT levels were much higher in fish from Whédos and Acadjas than from the Etsii and Fosu lagoon in Ghana (0.1–0.3 µg/kg) (Essumang et al. 2009). pp'-DDE (5.23 µg/kg) and pp'-DDT (3.65 µg/kg) levels in fish collected in the Bosomtwi lake (Darko et al. 2008) were in the same range as levels found in fish from Acadjas and Whédos in this study (2.8–3.1 and 0.34–1.2 µg/kg, respectively). pp'-DDE levels (0.05 µg/kg) were lower in *Channa marulius* collected in various streams of the Cauvery River in India (Begum et al. 2009) than in *P. obscura* from Whédos and Acadjas (14.8 and 15.2 µg/kg, respectively). In *P. obscura* from Nsawan near the Densu basin (Ghana) no pp'-DDE was detected. The pp'-DDT concentration in *P. obscura* measured at Nsawan (8.9 µg/kg) was in the range of the levels measured in the same species from Acadjas and Whédos. The concentration of α-endosulfan (71.3 µg/kg) measured in *P. obscura*

collected at Nsawan was much higher than the level measured in the same species from Whédos (1.24 µg/kg). High concentrations of HCH were measured in *P. obscura* collected at Nsawan near the Densu basin (16.1–35.2 µg/kg; Afful et al. 2010) or in fish from markets in the same area (0.10–17.65 µg/kg; Fianko et al. 2011), while it was not detected in the same fish species from the Acadja and of Whédos (Table 1).

In general, organochlorine pesticide concentrations in fish from the Acadjas and Whédos were in the same range as levels measured in fish from local markets in the Densu basin in Ghana (Fianko et al. 2011) or Abidjan in Côte D'Ivoire (Biego et al. 2010). Organochlorine pesticide concentrations in fish from the Acadjas and Whédos were lower than in fish from the Lagos Lagoon in Nigeria (Adeyemi et al. 2008). Except for endosulfan in *P. obscura*, fish from Lake Taabo in Côte D'Ivoire also contained higher pesticide levels than the fish from the Acadjas and Whédos analysed in this study (Roche et al. 2007).

Table 3 Risk assessment for human health of pesticide intake by consuming fish caught in Acadjas and Whédos along the Ouémé River in the Republic of Bénin

Fish name	<i>Clarias gariepinus</i>		<i>Protopterus annectens</i>		<i>Polypterus senegalensis senegalensis</i>		<i>Parachanna obscura</i>	
	Acadja	Whédo	Acadja	Whédo	Acadja	Whédo	Acadja	Whédo
Fish length (cm)	37.4	38	21	20	32.5	32	29.0	31
Fish weight (g)	234	238	39	39	191	191	150	152
Fish meat (g d.w.)	4.08	4.54	4.0	4	3.85	3.85	3.5	4.21
Fat content (%)	2.2	2.1	2.8	2.8	3.0	3.0	3.8	4.0
Pesticide concentration in fish (ng/g lipid)								
ΣDDT	324	212	140	72	280	287	1,185	756
α-endosulfan	120	7,926	24	102	23	678	158	31
Σdrin	4	9	10	8	5	35	57	<0.1
Lindane	3	7	<0.1	5	<0.1	6	<0.1	<0.1
Daily intake per one fish by an adult person of 60 kg (in ng/kg body weight)								
ΣDDT	0.48	0.34	0.26	0.13	0.54	0.5	2.6	2.1
α-endosulfan	0.18	12.6	0.045	0.19	0.044	1.3	0.35	0.087
Σdrin	0.006	0.014	0.019	0.015	0.010	0.067	0.13	<0.1
Lindane	0.0045	0.011	<0.1	0.0093	<0.1	0.012	<0.1	<0.1
Sum Toxic Units	0.00014	0.0023	0.00022	0.00020	0.00016	0.00095	0.0016	0.00023

Summed toxic units were obtained by relating daily intakes to the TDI defined by WHO (2004) of 10,000, 6,000, 100 and 5,000 ng/kg body weight, day for ΣDDT, ΣEndosulfan, Σdrin and lindane, respectively

To determine the potential risk of pesticide residues in fish for the human population living along the lower valley of the Ouémé River, the intake of Σ DDT, α -endosulfan, Σ drin and lindane by consuming Acadja and Whédo fish was estimated. For that purpose, it was assumed local people would eat one fish per day. Daily intakes were compared with TDI values reported by WHO (2004). Results are shown in Table 3. In all cases, the Σ TU values were far below 1.0, which indicates risk of pesticide exposure by eating fish from Acadjas and Whédos is limited. This confirms the findings of Yehouenou A. Pazou et al. (2006) who also found a low risk of pesticide intake by the consumption of fish from the Ouémé River in Bénin. It should be noted, however, that local fisherman may consume more fish, while residue levels may also be much higher in periods shortly after intensive pesticide application in agriculture. Further and more detailed research is needed to assess risk of pesticide exposure, taking into account frequency and timing of pesticide application and more realistic fish consumption rates.

Conclusion

This study shows that pesticide residue levels in fish might be influenced by the fishing technique. Organochlorine pesticide residues detected in fish collected from Acadjas and Whédos in general were similar but Whédo fish contained much higher endosulfan levels. A preliminary risk assessment suggested low risk for human health, but also indicated that further research is needed to fully characterize potential risks.

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