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Health Risks Associated With Pesticide Residues in Sediments, Fish, and Plants From the Ouémé Valley in the Republic of Bénin

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Abstract To determine possible human and environmental health risks, organochlorine pesticide residues were determined in vegetables grown in floodplains along the Ouémé River near Lowé in Bénin. Testing of vegetables found 13 pesticides with Σ DDT, α -endosulfan, Σ drin, and lindane being most important. The same pesticides were also detected in plants eaten by bovine cattle, sheep, and herbivorous fish. Human pesticide intake by vegetable consumption was compared with tolerable daily intake (TDI) values reported by the World Health Organization. Pesticide intake by fish consumption was estimated from residue levels in whole fish collected from the Ouémé River in 2004 and reported earlier. Fish consumption does not pose a risk for human health, but consuming vegetables that contain pesticide residues may lead to exceedance of

TDI values. Based on these findings, concerns are warranted, and more work is needed to understand the full exposure profile for the local population.

Environmental pollution by persistent organic pollutants (POPs) is of worldwide concern (United Nations Environment Programme 2010). POPs are organic compounds that are resistant to environmental degradation and generally have high lipophilicity. Due to their low degradation rates, high persistency, and high lipid solubility, POPs have high potential to accumulate in soils and sediments and to bioaccumulate or biomagnify in organisms and food chains (Borgå et al. 2001). POPs enter the environment due to general worldwide intensive production and use, among others, in industry and agriculture. POPs include industrial chemicals, such as polychlorinated biphenyls (PCBs) and dioxins, as well as a number of organochlorine pesticides (OPs), such as DDT and drins.

After understanding their long-term health risks, POPs were banned or their use severely restricted (UNEP 2010). The use of OPs for agriculture, however, has continued in some regions of the world, especially in developing countries. And for malaria control, the controlled use of DDT still is allowed in certain parts of the world (UNEP 2010). As a consequence, even in the developed nations that effectively enforced the ban on the use of OPs, residues are still detected in surface waters. Recent studies have indicated the presence of OP residues in surface waters, sediments, biota, and vegetations in Asia (e.g. Hung and Thiemann 2002; Pandey et al. 2011) and Africa (e.g. Ntow 2005; Yehouenou A. Pazou et al. 2006a, b; Darko et al. 2008; Mansour 2009; Musa et al. 2011).

In the Republic of Bénin, DDT was widely used in tsetse fly control programs, against malaria mosquitoes, and to

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control pests in agriculture. Despite its benefits for protecting humans from vector diseases, such as sleeping sickness and malaria, DDT was banned also in Bénin, mainly because of its human health risks. Some other OPs, especially endosulfan, are still used for cotton crop protection. Due to their low degradation rates and high persistency, high levels of endosulfan residues were identified in sediments and fish collected from the Ouémé River (Yehouenou A. Pazou et al. 2006a, b). OPs may reach the aquatic environment through direct run-off, leaching, washing of spray equipment, and careless disposal of empty containers. The concentrations of pesticide residues in surface sediments provide information on recent or past contamination. The determination of these pollutants in aquatic systems continues to be important in environmental toxicology because polluted waters and sediments may cause adverse biological effects on biota and consumers, including humans.

Lowé is a depressed floodplain area receiving water from the Ouémé River in the Republic of Bénin (Anthony et al. 2002). The valley is flooded during periods of high water. During the dry season, the water withdraws, and Lowé becomes a large agricultural area in which cotton, rice, cereals and vegetables are cropped with a high use of fertilizers and pesticides, especially for cotton culture. As a consequence, food produced in this area may possibly be contaminated with pesticides. This was confirmed by our earlier study, which showed increased pesticide levels in fish and sediments from the Ouémé River (Yehouenou A. Pazou et al. 2006a, b). No other data are available, however, on the possible human exposure to pesticides and the consequent health risks in the area.

This study, therefore, aimed at assessing the presence of OPs in plants collected or grown in the Lowé area and consumed by the local population. By comparing the estimated pesticide intake by fish (using data from our earlier study: Yehouenou A. Pazou et al. 2006b) and vegetable consumption with tolerable daily intake (TDI) levels described by the World Health Organization (2004) risk for the local population was assessed.

Materials and Methods

Study Area

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 Yehouenou A. Pazou et al. (2006a) for a map of the area. 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 for 6 months per year, usually from June to November, thus allowing (contaminated) sediment from upstream to settle.

Sampling

Approximately 1 kg of vegetables was collected from 10 locations in the river bank (100 g/location) by cutting with a knife when the river water had withdrawn. The vegetables were washed with drinking water to remove sand, wrapped in paper, and transported to the laboratory. Plant samples were dried in the laboratory temperature (27–30 °C) for 1 month. They were ground to powder and transported as dry powders to the Netherlands for analysis. Various fish species were caught with sparrow nets from the Ouémé River at Lowé. The fish were washed, stored in an ice chest at 4 °C, and transported to the laboratory. The fishes were weighted and identified using the key of Lévêque et al. (1990, 1992). Muscle tissues of each fish were collected, weighted, frozen, lyophilized, and stored at –20 °C before analysis. See Yehouenou A. Pazou et al. (2006b) for methods and results of the fish analysis.

Analytical Methods

All vegetable samples were analysed in the certified laboratory of the Hoogheemraadschap Hollands Noorderkwartier in Edam, The Netherlands. All solvents, as well as other chemicals used, were of analytical-grade quality to ensure purity. The freeze-dried and lyophilised plant samples (~5 g) were ground using a ceramic pestle and mortar. Samples were extracted with 200 ml of 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 twice with 500 ml deionized water to which 20 ml sodium chloride solution had been added. After washing, the petroleum ether fraction was dried with sodium sulphate and the volume reduced by evaporation in a Kuderna-Danish rotavapor at 75–80 °C.

For clean-up of the extract, an aluminium oxide column was used, which was eluted with petroleum ether. A second clean-up step was included using a column of deactivated silica gel and elution with petroleum ether. The extracts were concentrated to 2.0 ml under a gentle stream of nitrogen gas. Analysis took place by gas chromatography (Hewlett Packard 6890) using an electron capture detector equipped with an autosampler Hewlett Packard injector

7673 and a Chrompack 8753 CP-Sil-8 CB column (length 60 m, internal diameter 0.25 mm, film thickness 0.25 μm). Helium was used as the carrier gas. Injection temperature was 225 $^{\circ}\text{C}$, detector temperature 300 $^{\circ}\text{C}$, and column temperature stepwise increased from 90–275 $^{\circ}\text{C}$. OPs were identified by comparing their retention times with those of a standard mixture of PCBs and OPs. For calibration purposes, PCB155, mirex, and tetrachlorobenzene were used as internal standards, and a reference material was also analyzed. The limit of detection (LOD) for the OPs—including ΣDDT (pp'-DDE, op'-DDD, pp'-DDD, op'-DDT, pp'-DDT)—was 0.3 $\mu\text{g}/\text{kg}$ dry weight for the plant samples, and the limit of quantitation (LOQ) was 0.6 $\mu\text{g}/\text{kg}$ dry weight.

Quality-assurance measures applied in the laboratory 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. Recoveries of chlorinated pesticides in the reference material were between 80 and 110 % of certified concentrations.

Risk Assessment

For estimating risk due to pesticide intake by consumption of vegetables, the following approach was taken. TDI values as reported by the World Health Organization (2004) were used and expressed as nanograms per kilogram of body weight per day. These TDIs were multiplied with an estimated average human body weight of 60 kg to obtain tolerable intake (TI) levels in nanograms per day. These TI values were used to estimate tolerated residue limits in vegetables. For that purpose, the TI values were divided by the (worst case) consumption of 100 g dry weight of vegetable plants. Tolerated residue limits were estimated for each pesticide in each vegetable crop and expressed as nanograms per gram or micrograms per kilogram of dry weight of vegetable. This approach is in line with the way maximum residue limits for pesticides in food items are calculated (World Health Organization 1997). The toxic unit approach (Van Gestel et al. 2011) was adopted to enable summing the risk of the mixture of different pesticides detected in a food item. For that purpose, a toxic unit was defined as the pesticide concentration measured in a crop divided by the corresponding tolerated residue limit. The summed toxic unit combining the different pesticides was used as an indication of risk.

To assess the risk of pesticide intake by fish consumption by the local population, a slightly different approach was used. First, it was assumed that local people would eat 100 g dry weight of fish per day. By using the lipid content

of the fish, we estimated the corresponding intake of lipid (grams per day). Then we took the pesticide concentrations measured in fish from the Ouémé River near Lowé reported in our previous paper (Yehouenou A. Pazou et al. 2006b), which were expressed as nanograms per gram of lipid. Multiplying the lipid intake with the pesticide concentrations in nanograms per gram of lipid yielded the estimated daily intake for each pesticide in nanograms. Next, daily intake was normalized for an average human weighing 60 kg (World Health Organization 1997) to give intake values in nanograms per kilogram of body weight. These intake values were compared with the TDI values reported by the World Health Organization (2004). In addition, in this case the toxic unit approach was applied, with a toxic unit defined as the estimated daily intake of a pesticide by fish consumption divided by its TDI. Finally, the summed toxic unit was calculated to estimate the associated risk of consuming fish containing a mixture of different pesticides.

When the sum of the toxic units approached or exceeded a value of 1.0, this indicated a risk for the local population. By comparing the summed toxic units for fish and vegetable consumption, it could also be assessed which food item (fish or vegetable) posed the greatest risk for the local population in the Lowé area.

Results

Pesticide Residues in Vegetables and Plants Collected in the Lowé Floodplain Area

Vegetables and plants collected from the Lowé floodplain area and from the Ouémé River bank were contaminated with 13 different pesticides (Table 1). Pesticide concentrations ranged from the detection limit (0.6 $\mu\text{g}/\text{kg}$ dry weight) to 502 $\mu\text{g}/\text{kg}$ dry weight. DDT and its metabolites, dieldrin and α -endosulfan, were identified in all plants collected. Among the plants analyzed, four are eaten by humans and the other three by cattle and fish. Vegetables eaten by humans are *Amaranthus hybridus* (commonly called smooth amaranth, smooth pigweed, red amaranth, or slim amaranth), *Solanum macrocarpon* (African eggplant), *Vernonia amygdalina*, and *Corchorus olitorius*. *A. hybridus* and *S. macrocarpon* are vegetable plants eaten by African people the way spinach is eaten as a vegetable by European people. These vegetables are eaten daily by the local population. *V. amygdalina* is a small shrub commonly called “bitter leaf” because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. *C. olitorius* is used to vary the meal. *Leersia hexandra* is eaten by sheep, *Celosa argentea* by cows, and *Ipomea fistulosa* by herbivorous fish. Of the

vegetables eaten by humans, *A. hybridus* had the highest levels of Σ DDT (1,578 $\mu\text{g}/\text{kg}$ dry weight) followed by *C. olitorius* (1,384 $\mu\text{g}/\text{kg}$ dry weight), which also had the highest levels of endosulfan. Dieldrin and lindane were identified in all plants consumed by humans, and there was a high a level of lindane (444 $\mu\text{g}/\text{kg}$ dry weight) in *C. olitorius*. Of the plants eaten by animals, *I. fistulosa* had the highest levels of Σ DDT followed by *L. hexandra* (Table 1).

Risk Assessment

Risk assessment for pesticide intake by vegetable consumption was performed by comparing measured pesticide concentrations with estimated tolerated residue levels for the different pesticides in vegetable crops. Table 2 lists this approach and shows the summed tolerated residues levels in vegetables. The concentrations of the individual pesticides did not exceed the tolerated residue levels. However, the summed toxic units did exceed 1.0 in *A. hybridus* ($\Sigma\text{TU} = 1.1$) and *C. olitorius* ($\Sigma\text{TU} = 1.5$), suggesting that total pesticide intake by consuming vegetables may exceed maximum tolerated levels.

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 three groups of pesticides (Σ DDT, Σ endosulfan, and dieldrin) from fish consumption was estimated. For that purpose, data from our previous study (Yehouenou A. Pazou et al. 2006b) in the Lowé basin were used. In that study, different fish species were collected and analysed. Results are listed in Table 3. Based on a consumption of 100 g dry weight fish meat/day, lipid contents, and pesticide concentrations expressed on a lipid basis, daily intake was estimated. By comparison with TDI values for different pesticides, the summed risk was calculated and expressed as toxic units. TDI values for Σ DDT, Σ endosulfan, and dieldrin are 10, 6, and 0.1 $\mu\text{g}/\text{kg}$ body weight/d, respectively (World Health Organization 2004).

For all fish species, the sum of toxic units (ΣTU) was far lower than 1.0, thus indicating that the risk of pesticide intake by fish consumption is low.

Discussion

The residue levels of endosulfan in vegetables can be explained from its use as an insecticide in agriculture; the same counts for the somewhat increased concentration lindane in *C. olitorius*. The presence of DDT and its metabolites at increased levels in all vegetable plants suggests illegal use of this pesticide in the area. This is confirmed by the dominance of DDT in the Σ DDT concentrations (Gitahi et al. 2002; Dem et al. 2007; Darko et al. 2008). In all cases, DDT levels were as high as or even greater than the levels of the more persistent metabolites DDE and DDD. When ratios of DDT/DDE or DDT/DDD are high, this suggests that DDT has recently been sprayed in the area (Gitahi et al. 2002; Dem et al. 2007; Darko et al. 2008). Pesticide residue levels in vegetables from Egypt were reported to be lower than the ones found in this study, exceeding the maximum residue limits only in few cases (Mansour 2009). Concentrations reported for beet roots, onions and potatoes from organic farming in Egypt were 0.23–0.66, 1.78–2.82, 0.60–0.84, and 0.30–0.70 $\mu\text{g}/\text{kg}$ fresh weight for endosulfan, Σ DDT, lindane, and Σ drins, respectively (Mansour 2009). Assuming a dry-weight content of 10 and 20 % for onions and potatoes, respectively, these levels correspond with endosulfan, Σ DDT, lindane, and Σ drin concentrations of 1.2–6.6, 8.9–28.2, 3.0–8.4, and 1.5–7.0 $\mu\text{g}/\text{kg}$ dry weight, respectively. Σ DDT, Σ HCH, and Σ drin levels in vegetables from Cambodia were in same range as the ones found in this study (Wang et al. 2011). A rather wide range of pesticide concentrations was found in *Caspsicum annum* collected from Nigerian markets: HCH residues dominated

Table 1 Pesticides residue levels ($\mu\text{g}/\text{kg}$ dry weight) in vegetables and plants collected in the floodplain of the Ouémé River at Lowé, Republic of Bénin

Vegetable species	op'-DDE	pp'-DDE	op'-DDD	pp'-DDD	op-DDT	pp-DDT	α -endo	aldrin	dieldrin	endrln	lindane	Hbenz	epH
<i>A. hybridus</i>	17	225	155	387	292	502	232	<5	34	<5	97	3	7
<i>S. macrocarpum</i>	8	127	64	145	115	188	138	<1	28	<5	21	3	4
<i>V. amygdalina</i>	6	134	40	161	75	245	68	<1	12	<2	7	3	2
<i>C. olitorius</i>	17	245	128	274	270	450	386	<2	45	<9	444	5	8
<i>L. hexandra</i>	14	234	109	248	198	401	179	9	42	<23	32	5	14
<i>I. fistulosa</i>	19	295	195	565	351	671	171	<2	36	<2	23	3	4
<i>C. argentea</i>	8	153	92	156	121	280	116	<2	22	<6	<6	<2	<2

α -endo α -endosulfan, *endr* endrin, *Hbenz* hexachlorobenzene, *epH* epoxy-hexachlorocyclohexane

Table 2 Estimation of the potential risk of pesticide intake by vegetable consumption for the local population of the Lowé valley, Republic of Bénin

	ΣDDT	α-endosulfan	Σdrins	Lindane	ΣHCH-epoxy	ΣTU
TDI (µg/kg body weight/d)	10	6	0.1	5	0.1	
Tolerated residue levels (µg/kg dw)	6,000	3,600	60	3,000	60	
Pesticide levels in vegetable crops (µg/kg dw)						
<i>A. hybridus</i>	1,578	232	34	97	10	1.1
<i>S. macrocarpum</i>	647	138	28	21	7	0.74
<i>V. amygdalina</i>	661	68	12	7	5	0.41
<i>C. olitorus</i>	1,384	386	45	444	13	1.5

Tolerated residue levels in crops were calculated assuming a vegetable consumption of 100 g dry weight per day by an adult person weighing 60 kg using TDI values established by the World Health Organization (2004). ΣTU were obtained by relating pesticide residue levels measured in the different vegetable crops with the tolerated residue levels and summing the resulting TU values for the different pesticides measured in each crop

Table 3 Estimation of the potential risk of pesticide intake by fish consumption for the local population of the Lowé valley, Republic of Bénin

Fish species	<i>Synodontis nigrita</i>	<i>Schilbe intermedius</i>	<i>Chrysichtys auratus</i>	<i>Hyperopisus bebe</i>	<i>Clarias ebriensis</i>	<i>C. gariepinus</i>
Fat content (%)	4.7	18	3.7	2.6	2.9	4.2
Pesticide concentration in fish meat (ng/g lipid)						
ΣDDT	571	1,191	1,239	814	242	891
ΣEndosulfan	87	215	121	85	9	26
Dieldrin	7	9	4	0	0	10
Daily intake per 100 g dry fish by an adult person of 60 kg (ng/kg body weight)						
DI ΣDDT	44.7	357	76.4	35.3	11.70	62
DI ΣEndosulfan	6.82	64.5	7.46	3.68	0.435	1.82
DI Dieldrin	0.548	2.70	0.247	0.00	0.00	0.70
ΣTU	0.011	0.074	0.011	0.0041	0.0012	0.014

It was assumed that fish consumption amounted to 100 g dry weight/d. Fat content and pesticide residue levels in fish were taken from Yehouenou A. Pazou et al. (2006b). Daily intake values were calculated by multiplying pesticide concentrations (ng/g lipid) with the amount of fat (g) per 100 grams of dry weight of fish and dividing by 60 kg body weight. TUs were obtained by relating daily intakes to the TDI values as defined by the World Health Organization (2004) of 10,000, 6,000, and 100 ng/kg body weight/d for ΣDDT, Σendosulfan, and dieldrin, respectively. Combining the toxic units for these three pesticides yielded ΣTU

with levels as high as 1,450–2,340 µg/kg, whereas pp'-DDT and pp'-DDE levels were 64–99 and 7–105 µg/kg, respectively (Benson and Olufunke 2011).

Mansour (2009) reported aquatic plants from Egypt to contain ΣDDT, lindane, and hexachlorobenzene levels of 6.1–6.6, 0.14–0.65, and 1.6–7.1 µg/kg fresh weight, respectively. These concentrations correspond with levels of ~30–33, 0.7–3.3, and 8.0–36 µg/kg dry weight, respectively, when assuming a plant dry-weight content of ~20 %. Except for hexachlorobenzene, these levels are much lower than the ones found in this study.

This study showed considerable risk for human health due to pesticide residues especially in plants commonly consumed by the local people in Benin, in some cases with ΣTU >1.0. When considering that cattle and sheep are consuming greater amounts of plants on a body-weight basis, the pesticide intake will probably also lead to

increased pesticide levels in their tissues. Although this does not necessarily damage the health of cattle and sheep, pesticides accumulating in their body may result in increased levels in products derived from these animals, such as meat and milk. This was also shown by Mansour (2009), who reported increased levels of OPs in birds and in cattle, camel, and sheep and their products (meat, milk, cheese) from Egypt, suggesting exposure through feeding on contaminated fish and vegetation. The exposure of these animals, which are common in the African diet therefore may add to human exposure because pesticides accumulated from food may be transferred to consumers of meat and milk.

The increase pesticide levels in plants eaten by fish might also contribute to increased pesticide levels in fish. Interestingly, exposure to fish seemed to be negligible compared with the human health risk of pesticide exposure

through the consumption of vegetables. For humans, the direct route of exposure to vegetables therefore seems much more important than indirect exposure to plant-eating fish.

The results of this study suggest a risk of pesticide exposure from the consumption of contaminated vegetable crops. Exposure through this route by far exceeds that from eating fish. As a consequence, risk of pesticide intake by fish consumption is negligible for the local population in the Lowé area, which confirms the conclusions of our earlier study (Yehouenou A. Pazou et al. 2006b).

Conclusion

This study shows that the Ouémé floodplain area at Lowé was contaminated by OPs that accumulate in the food chain and therefore may pose a risk to human health. Pesticides are still used in the area with expansion of agricultural activities. Human health risk of pesticide intake by fish consumption is low, but pesticide intake by vegetable consumption is high and in some cases exceeds tolerable intake levels. When pesticides also accumulate in cows and sheep feeding on plants grown in the area, they may act as an additional source of pesticide exposure for humans consuming milk and meat. This study shows the need for further and detailed assessment of the human health risks of pesticide residues in the Ouémé River floodplain areas of Bénin.

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