PRESENCE OF ORGANOPHOSPHATE INSECTICIDES IN FISH OF THE AMAZON RIVER

Nicolas SOUMIS1*, Marc LUCOTTE1, Delaine SAMPAIO2, Diane CRUZ ALMEIDA2, Dalie GIROUX3, Silmara MORAIS2, Pierre PICHET4

ABSTRACT – Trace levels of three organophosphate insecticides (OPI) were detected in eight fish species from the region of Santarém, State of Pará, Brazil. Individual concentrations of OPI in fish ranged from less than the detection limit to 2.1 ppb. Mean concentrations of chlorpyrifos, malathion, and methyl-parathion were 0.3 ± 0.3, 0.1 ± 0.1, and 0.3 ± 0.3 ppb, respectively. Pellona flavipinnis, the largest and fattest piscivorous species analyzed, was the most contaminated. Since an inhabitant of this Amazonian region consumes 220 g of fish per day on average, ingested doses of chlorpyrifos, malathion, and methyl-parathion may reach up to 308, 220, and 462 ng·d⁻¹, respectively. Compared to acceptable daily intakes (ADI), quantities of OPI absorbed via fish consumption on a daily basis are far below deleterious levels. We estimated that even considering the highest OPI contents detected, the average daily fish consumption of an adult of 60 kg would have to increase by ca. 1 950, 5 450, and 2 600 times to reach ADI of chlorpyrifos, malathion, and methyl-parathion, respectively. Neither fish diet nor fish lipid content enabled us to completely explain the interspecific differences observed.

Key-Words: Brazilian Amazon, fish contamination, organophosphate insecticides, acceptable daily intake (ADI), human alimentation.

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INTRODUCTION

We previously performed a study of pesticide consumption in the region of Santarém, an important urban center of the Lower Amazon, Brazil (Soumis et al., 2000). Based on surveys among several local crop producers and agricultural suppliers, this study established that besides other pesticides such as fungicides and herbicides, organophosphate insecticides (OPI) were the most common compounds into the pesticide arsenal of this region, their consumption reaching ca. 2 000 kg·year\textsuperscript{-1} (active ingredients). The importance of these insecticides is also reflected by pesticide consumption figures for Brazil gathered by the Food and Agriculture Organization (FAO) that indicate an upward trend in the use of OPI between 1991 (1 850 tons) and 1995 (5 384 tons)\textsuperscript{1}. These insecticides are currently used on crops established on floodplains (várzeas) of the Amazon River. Várzea designate the portion of land along rivers which is flooded during the rainy season. Annual floods carry large amounts of nutrient-rich Andean sediments which deposite on várzeas, a fertilizing phenomenon that ensures an enhanced agricultural potential for these environments (Furch, 1997; Ohly and Junk, 1999; Zarin, 1999).

Apart from supporting agriculture, várzeas also represent important fishing sites in view of their abundant and diverse ichthyofauna (Ruffino, 1996; Cerdeira et al., 1997; Ruffino et al., 1998). Now the multiple use of this environment may be problematic since the contamination of fish by OPI is likely to occur for several reasons. First, várzea crops are located in the vicinity of aquatic ecosystems. Hence, wind drift, volatilization, and frequent heavy rainfalls in the Amazon Basin (IBGE, 1977, in Salati, 1985; Ohly, 1987) may cause OPI to reach streams and lakes nearby crops (Racke, 1992). Second, OPI remain in their original form long enough (from several days to a few months) to allow them to move away from their application site. Third, many OPI are hydrophobic compounds; in aqueous media, they have much more affinity for the biological compartments (Sabharwal and Belsare, 1986; Cowgill et al., 1991).

Basic information on the pesticide burdens in fish remains of first concern in the Lower Amazon as fisheries represent one of the principal economic activities in this region, and as fish is the main source of animal proteins in the diet of local populations (Saint-Paul and Bayley, 1979; Ferreira et al., 1996; Isaac et al., 1996). According to our knowledge, very few studies has yet been conducted in order to assess the concentration of agricultural pesticide in the Amazonian ichthyofauna (Torres et al., 2002). Considering a situation which may jeopardize the health of the local populations and alter an important pillar of the regional economy, the present study constitutes a preliminary assessment of the ichthyofauna contamination by three OPI commonly used on várzea crops: chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate; CAS : 2921-88-2; $K_{ow}$ : 158 489), malathion (O, O-dimethyl S-(1, 2-dicarbethoxyethyl) phosphorodithioate; CAS : 121-75-5; $K_{ow}$ : 776), and methyl-parathion (O, O-dimethyl O-(4-nitrophenyl) phosphorothioate; CAS : 298-00-0; $K_{ow}$ : 1 288). The following objectives were pursued: 1) to obtain a portrait of the fish contamination by OPI in the region of Santarém; 2) to compare the results obtained with acceptable daily intakes\textsuperscript{2} (ADI) in order to determine if the daily doses absorbed by local populations reach a theoretically critical level; 3) to verify the existence of significant interspecific differences in contamination levels and to assess some biological factors that may be responsible for such differences.

\textsuperscript{1}According to FAOSTAT, a FAO’s database available on the Web (http://apps.fao.org/page/collection). Data from FAO are only available from 1991 to 1995.

\textsuperscript{2}Acceptable daily intake was defined by the World Health Organization (WHO) as the daily intake of a chemical which, during an entire lifetime, appears to be without appreciable risk on the basis of all known facts at the time (WHO, 1962, in Klaassen and Eaton, 1991). ADI is expressed in mg of chemical by kg of the consumer body.
STUDY AREA

Located at the confluent of the Tapajós and the Amazon Rivers in the State of Pará, Santarém (2° 25’ S, 54° 43’ W) is the principal urban center of the Lower Amazon. This region was selected for many reasons. First, an important concentration of agricultural activities take place around Santarém (Scatena et al., 1996). Second, this region also encompasses one of the greatest concentrations of várzeas of the Brazilian Amazon. Third, as the largest fishing port of the Lower Amazon, Santarém receives the catches from 14 municipalities along the Amazon River (Isaac et al., 1996; Ruffino et al., 1998). It is thus possible to collect at Santarém’s fish markets specimens coming from the whole region.

MATERIAL AND METHODS

The sampling campaign was conducted by the end of September of 1998, during the low water season. At that time of year, as river recedes to its lowest level (Fig. 1), the whole várzeas are free of water and agricultural activities attain its optimum. Consequently, the highest pesticide consumption is expected to be found during this season. One hundred and twenty fresh specimens, equally distributed between eight species (Tab. 1), were bought from a fish market located in the port of Santarém during a five-day sampling. According to salemen and fishermen, specimens collected were known to belong from the region.

Figure 1 - Fluctuations of water level for 1998. Measurements were made twice a day at the Portobrás’ wharf in Santarém, located at the confluence of the Amazon and Tapajós Rivers (Data from Delegacia da Capitania dos Portos dos Estados do Pará e Amapá em Santarém).

Table 1 - Names, diet and lipid content of fish species sampled from the market of Santarém.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Common name (Brazilian)</th>
<th>Diet</th>
<th>Lipid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cichla monoculus</td>
<td>tucunaré</td>
<td>piscivorous</td>
<td>0.5% (low)</td>
</tr>
<tr>
<td>Colossoma macropomum</td>
<td>tambaqui</td>
<td>herbivorous</td>
<td>0.2% (low)</td>
</tr>
<tr>
<td>Liposarcus pardalis</td>
<td>acarí bodó</td>
<td>detritivorous</td>
<td>0.1% (low)</td>
</tr>
<tr>
<td>Mylossoma duriventre</td>
<td>pacu comum</td>
<td>herbivorous</td>
<td>7.0% (high)</td>
</tr>
<tr>
<td>Pellona flavipinnis</td>
<td>apapá branco</td>
<td>piscivorous</td>
<td>6.4% (high)</td>
</tr>
<tr>
<td>Plagioscion squamosissimus</td>
<td>pescada branca</td>
<td>piscivorous</td>
<td>2.0% (intermediate)</td>
</tr>
<tr>
<td>Pygocentrus nattereri</td>
<td>piranha caju</td>
<td>piscivorous</td>
<td>1.2% (intermediate)</td>
</tr>
<tr>
<td>Schizodon fasciatum</td>
<td>aracu comum</td>
<td>herbivorous</td>
<td>2.0% (intermediate)</td>
</tr>
</tbody>
</table>
In the laboratory, filets free of scales, skin, and bones were drawn from fish so as to be frozen (-20 °C) in amber-glass bottles with teflon-septum caps. Extraction of OPI residues from the filets was carried out according to methods 304-E1 (extraction) and 304-C2 (cleanup) of the American Food and Drug Administration (FDA) suitable for fatty matrices. This solid/liquid multiresidue extraction procedure allows for the simultaneous extraction of several nonpolar pesticides. As a result, only parental OPI were quantitatively recovered since it is impossible to extract polar metabolites of OPI with this procedure.

We slightly modified the FDA methods to better fit our needs. Hereafter are the few modifications we brought. For the extraction step, 30 g (wet weight) of fish filet were ground with 60 g of anhydrous sodium sulfate (Na$_2$SO$_4$). We determined the quantity of fish according to fat content (Junk, 1985) to avoid overwhelming the retention capacity of the cleanup column. For the concentration step, we used a rotary evaporation system instead of the Kuderna-Danish concentrator recommended by the FDA since we achieved better results with the former device. For the cleanup step, we followed instructions for the methylene chloride elution system, although we only used eluants "2" and "3" (50% methylene chloride, 0,35% acetonitrile, 49,65% hexane (v/v/v) and 50% methylene chloride, 1,5% acetonitrile, 48,5% hexane (v/v/v), respectively). As we collected all of our analytes through these two eluants, the eluant "1" proposed by FDA was useless in our case. All reagents were ACS grade; they were concentrated and tested for contamination beforehand.

Cleaned extracts were concentrated to 2 ml prior to analysis. OPI analyses were conducted with a Varian 3800 gas chromatograph (GC). Our system was equipped with an autosampler (Varian 8200), a splitless glass insert (0.5 mm ID), a SPB-5 fused silica capillary column (Supelco, 30 m length, 0,25 mm ID and 0,25 µm film) protected by a deactivated and uncoated fused silica guard column (Siltek, 5 m length, 0,25 mm ID), and provided with a thermoionic specific detector (TSD). TSD response was monitored during routine analyses by external standards. Injector and TSD temperatures were set at 230 and 300 °C, respectively. The temperature programming of the GC oven was as follow: initial temperature, 80 °C, initial hold time, 120 s; ramp rate of 40 °C·min.$^{-1}$ to 150 °C, hold time, 60 s; ramp rate of 4 °C·min.$^{-1}$ to 200 °C, hold time, 300 s; and ramp rate of 40 °C·min.$^{-1}$ to 290 °C, final hold time, 360 s; total run time, 30,5 min. Helium was the carrier gas (1,5 ml·min.$^{-1}$) while nitrogen was the make-up gas (28,5 ml·min.$^{-1}$). Each analysis was made in triplicate, with an injection volume of 2 µl.

Recovery rate (± standard deviation) for each OPI was 110% ± 10%. Detection limits for chlorpyrifos, malathion, and methyl-parathion were 2,45, 1,65, and 3,20 ppm (concentrations of the extract injected in the GC), respectively. Concentrations of insecticides in fish (wet weight) were calculated separately in order to compare each one to its own ADI. Undetected compounds were assumed to be present at half the detection limit (see above) of the GC rather than absent from specimens.

In order to further our understanding of the ichthyofauna contamination, we tested some biological factors that may influence OPI levels in specimens. We first checked for the existence of interspecific differences. Then, we explored two factors (diet and lipid content) which might explain these interspecific differences. Determination of diet (Tab. 1) was made according to literature (Goulding and Carvalho, 1982; Soares et al., 1986; Ruffino and Isaac, 1995; Junk et al., 1997; Ferreira et al., 1998). Lipid contents were estimated according to Junk (1985), by using figures for the low water season (Tab. 1). For all parameter tested, we used Kruskal-Wallis’ tests and a set of Wilcoxon-Mann-Whitney’s tests every time a Kruskal-Wallis test showed a significant difference. In addition, Kendall’s rank correlation tests were used to verify the existence of a relation between the lipid content and the OPI level in filets.

These methods are found in the Pesticide Analytical Manual, volume 1. This document is available on the Web at the following URL: http://vm.cfsan.fda.gov/~rfr/pami3.html (see chapter 3).
RESULTS

Description of the fish sample

Our results indicate that OPI found in the specimens analyzed are at trace levels. As depicted by the histograms of Figure 2, which illustrate distributions of the 120 specimens according to their OPI concentrations, most specimens are very slightly contaminated albeit a restricted few show much more higher concentrations. Moreover, the exponential pattern of distributions clearly justifies the use of nonparametric tests for further statistical data analysis.

Table 2 lists some statistical parameters for the whole sample and for each species. Considering the whole sample, the majority of specimens are contaminated to a detectable level by chlorpyrifos and methyl-parathion (59.2% and 68.3%, respectively), although this is not true in the case of malathion (only 25.8% detected). Furthermore, 20% of the sample show no detectable contamination by any of the three OPI. When specimens are divided according to their species (Tab. 2), it appears that the occurrence and the difference between the particular mean and median vary from one species to another. These observations suggest specific trends, which become more obvious (2 words) from the boxplots of Figure 3.

Daily doses absorbed by populations and comparison to acceptable daily intakes

According to Cerdeira et al. (1997), the average fish meat consumption of a riverine population living near Santarém is about 220 g·d⁻¹ inhabitant (a point is missing). Although it is likely that urban populations consume a smaller quantity of fish per day (Shrimpton and Giugliano, 1979; Smith, 1979; Amoroso, 1981), we extrapolated this figure to the whole region of Santarém. Table 3 estimates OPI daily doses ingested by local populations via their fish consumption.

Acceptable daily intakes (ADI) for chlorpyrifos, malathion, and methyl-parathion...
Soumis et al. are fixed at 0.01, 0.02, and 0.02 mg·kg$^{-1}$, respectively (Lu, 1995). Assuming a hypothetical body weight of an adult consumer of 60 kg$^4$, we calculated that critical daily doses for chlorpyrifos, malathion, and methyl-parathion are 0.6, 1.2, and 1.2 mg·d$^{-1}$, respectively. Considering the latter figures, it is obvious that even the maximum daily doses to which local populations are exposed (Tab. 3) are far smaller than those required to reach ADI. In fact, daily fish consumption would have to increase by ca. 1,950, 5,450, and 2,600 times to reach ADI of chlorpyrifos, malathion, and methyl-parathion, respectively. In other words, with such "security margins", probabilities of attaining the critical doses stated above with fish from the sampled market are infinitesimal.

Table 2 - Statistical parameters for the whole sample and for each species.

<table>
<thead>
<tr>
<th>Group</th>
<th>Size</th>
<th>Insecticide</th>
<th>Range (ppb)</th>
<th>Median (ppb)</th>
<th>Mean (ppb)</th>
<th>Std dev. (± ppb)</th>
<th>Occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole sample</td>
<td>120</td>
<td>chlorpyrifos</td>
<td>0.1 - 1.4</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>59.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>0.0 - 1.0</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 2.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>68.3</td>
</tr>
<tr>
<td>C. monocus</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 1.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>60.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>0.0 - 0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>53.3</td>
</tr>
<tr>
<td>C. macropomum</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 1.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>0.0 - 0.7</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.1</td>
<td>86.7</td>
</tr>
<tr>
<td>L. pardalis</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 0.9</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>&lt; DL - 0.4</td>
<td>&lt; DL</td>
<td>&lt; DL</td>
<td>0.1</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.7</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>80.0</td>
</tr>
<tr>
<td>M. duriventre</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>&lt; DL - 0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>0.1 - 0.7</td>
<td>0.3</td>
<td>0.3</td>
<td>0.2</td>
<td>60.0</td>
</tr>
<tr>
<td>P. flavipinnis</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.2 - 1.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>0.1 - 1.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.4 - 2.1</td>
<td>0.8</td>
<td>0.9</td>
<td>0.5</td>
<td>100.0</td>
</tr>
<tr>
<td>P. squamosissimus</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 1.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>&lt; DL - 0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>53.3</td>
</tr>
<tr>
<td>P. nattereri</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>&lt; DL - 0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.5</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>60.0</td>
</tr>
<tr>
<td>S. fasciatum</td>
<td>15</td>
<td>chlorpyrifos</td>
<td>0.1 - 1.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>malathion</td>
<td>&lt; DL - 0.3</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>53.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>methyl-parathion</td>
<td>0.1 - 0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>53.3</td>
</tr>
</tbody>
</table>

a: Determines the ratio of specimens in which the compound has been positively detected.
< DL : Smaller than the detection limit

are fixed at 0.01, 0.02, and 0.02 mg·kg$^{-1}$, respectively (Lu, 1995). Assuming a hypothetical body weight of an adult consumer of 60 kg$^4$, we calculated that critical daily doses for chlorpyrifos, malathion, and methyl-parathion are 0.6, 1.2, and 1.2 mg·d$^{-1}$, respectively. Considering the latter figures, it is obvious that even the maximum daily doses to which local populations are exposed (Tab. 3) are far smaller than those required to reach ADI. In fact, daily fish consumption would have to increase by ca. 1,950, 5,450, and 2,600 times to reach ADI of chlorpyrifos, malathion, and methyl-parathion, respectively. In other words, with such "security margins", probabilities of attaining the critical doses stated above with fish from the sampled market are infinitesimal.

**Biological factors influencing The contamination (2 words) level of fish by OPI**

According to Kruskal-Wallis’ tests, interspecific differences that we already suspected from boxplots of Figure 3 proved to be very highly significant for all three OPI. Further analysis using Wilcoxon-Mann-Whitney’s tests indicated that these differences are caused by 1) *P. flavipinnis* (higher than all other species) and *P. squamosissimus* (higher than *L. pardalis*, *P. nattereri*, and *S. fasciatus*, but lower than *P. flavipinnis*) in the case of chlorpyrifos; 2) *P. flavipinnis* (higher than all other species, except *C. macropomum* and *S. fasciatus*) and *L. pardalis* (lower than all other spe-

$^4$Based on a study made in a small community along the Tapajós River (Brasilia Legal), the average Amazonian adult female weight is 58 kg and the average adult male weight is 63 kg (D. Mergler, personal communication).

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DISCUSSION

Choice of the study period and representativeness of the sample

Being tightly linked to the seasonal water cycle of the Amazon River, agriculture on várzeas is subjected to wide fluctuations in the course of a year (Fig. 1). During the high...
water season, most várzea soils are flooded and agricultural activities are scarce, being often confined to small gardens on stilts (Padoch and Pinedo-Vasquez, 1999). However, as heavy rainfalls are frequent during this period of the year, runoff with the few pesticides applied is prone to occur. On the contrary, during the low water season, agriculture and pesticide applications reach their optimum as plenty of soil is available for crops. However, because precipitations are less abundant during this season, pesticide runoff may be lower in comparison with the high water season.

In a comprehensive study of pesticide contamination in fish, it would have been relevant to conduct several sampling campaigns along the year in order to assess each set of environmental and agricultural conditions. However, keeping in mind that we conducted a preliminary study on this issue, we rather focused on a single period we considered the most critical. For several reasons, we estimated that the low water season could be that critical period during which fish contamination may be at its highest. First, and as explained above, it is expected to find the highest pesticide consumption at that time of the year. Second, the averaged daily precipitations in September are about 3 mm (Xie and Arkin, 1996, in Zeng, 1999). Hence, although fish contamination is more likely to come from wind drift and volatilization during that time, insecticides runoff is also likely to occur. Third, as fish populations are more confined due to the drying-up of some aquatic habitats, fishing activities increase during the low water season (Ohly and Junk, 1999). Probabilities are thus higher to consume a contaminated specimen during that period of the year.

The eight species collected cannot give an exhaustive picture of the Lower Amazon’s ichthyofauna biodiversity. Indeed, more than sixty fish species are found in the several markets of Santarém (Isaac et al., 1996; Ruffino et al., 1998). Nevertheless, only ten species out of those sixty account for 80-86% of the catches landed in Santarém (Isaac et al., 1996; Ruffino et al., 1998). Since the eight species collected during this study are among the latter, we can reasonably assume that our sample is representative of the commercial species currently found in Santarém during the low water season.

**Sanitary hazards linked to fish consumption**

As far as OPI are concerned, the daily fish consumption does not appear to represent a health hazard for the Amazonian populations since detected levels of these compounds are far below their ADI. Several considerations...
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Determination of biological factors affecting the contamination levels in fish

Although we found that the contamination level varies from a species to another, the exact causes of this situation still remain unknown. On the one hand, statistical tests have shown that no difference was induced by the diet in the case of chlorpyrifos and methyl-parathion. However, the same biological factor seems to induce a very highly significant difference in the case of malathion. While this difference is due to the detritivorous group represented by *L. pardalis* alone, it is yet dubious to draw any conclusion from a single species as differences could arise from other specific factors. On the other hand, statistically significant differences caused by the lipid content also remain ambiguous. By correlating the lipid contents of fish with their contamination levels, we expected to find a concomitant augmentation of both variables, a phenomenon which was assessed by Kendall’s rank correlation tests. However, these tests have shown but a weak relation between those variables. Moreover, only the high lipid content group distinguished itself from the other groups. It appears that the differences are mainly due to *P. flavipinnis* since *M. duriventre* – the other species comprised in the high-lipid group – presents relatively low OPI levels in spite of its higher lipid content.

A last limit still deserves attention. Each insecticide was individually compared to its particular ADI. Now, considering that most specimens are contaminated with more than one compound and given the existence of synergistic or additive effects between some OPI (Gallo and Lawryk, 1991), we must be careful when drawing conclusions from norms established for a single insecticide. However, one may argue that thanks to the wide security margins we defined, it is unlikely that additive or synergistic effects of OPI present in fish reach a deleterious level. Nevertheless, the concomitant absorption of pesticides from other alimentary sources such as fruits or vegetables may remain dangerous. In a similar way, the possible presence of other neurotoxic chemicals in fish – particularly organomercuric compounds (Lebel *et al.*, 1997) – may cause unsuspected synergistic effects which are not covered by this study.

The absence of any significant difference caused by the diet in the case of chlorpyrifos and methyl-parathion may suggest some indications about the phase and the way these OPI are introduced in the aquatic environment, and as to how they contaminate the ichthyofauna.
Despite the interest of these prospective leads, we remain careful regarding the validity of the following hypotheses mainly because of the low contamination levels and the relatively high number of specimens with undetected OPI.

Two phenomena may explain the similar contamination levels of the different dietary groups: 1) an even distribution of contaminants among all food sources for fish, which would allow equal body burdens via biomagnification; 2) the presence of OPI in the water column which may be readily absorbed by bioconcentration. Both phenomena imply the presence of dissolved chlorpyrifos and methyl-parathion, forms unlikely to be introduced in water by runoff or leaching from treated soils since these two insecticides are known to be well adsorbed onto soil particles (Wauchope, 1978; Agnihotri et al., 1981). Now there are only two possible ways to find dissolved OPI in the water column. One means is through volatilization, atmospheric transport, and precipitations (Racke, 1992; Bidleman, 1999; Van Dijk and Guicherit, 1999). These routes play a major role in OPI transportation, especially during a period of low rainfall. We can also suspect some bioconcentration arising from a slow desorption process of OPI from the sediments, which constitute an important sink for these contaminants associated with soil particles (Hughes et al., 1980; Sabharwal and Belsare, 1986; Bhushan et al., 1997).

Between both hypotheses on bioaccumulation stated above, bioconcentration seems to be the principal route through which OPI are absorbed by fish. Indeed, OPI are not transferred along the food chain to a great extent because they are easily decayed by the in vivo digestive processes of animals (IPCS, 1986), plant metabolism (Sabharwal and Belsare, 1986; Racke, 1992), and even chemical/microbial processes in sediments and flooded soils (Sabharwal and Belsare, 1986; Adhya et al., 1987), thus tempering biomagnification processes. Moreover, there is some evidence that bioconcentration is the most relevant route of contaminant exposure in the aquatic habitat (Chiou et al., 1977; Bruggeman et al., 1981).

CONCLUSION

Focusing on the agricultural pollution of the ichthyofauna by OPI, this study has shown that detected concentrations of some of these insecticides in fish are several times lower than their respective ADI. It thus appears that the contamination of the ichthyofauna by OPI is unlikely to occur to a problematic extent in the region of Santarém. Pellona flavipinnis, a large and high lipid-content piscivorous species, was the most contaminated fish of our sample. Still, the exploratory and partial nature of this study should not be overlooked. Concerning the assessment of the contamination by other classes of pesticides or by OPI metabolites, we allowed ourselves to speculate on the wide security margins we determined. Nevertheless, we insist on the fact that either this statement or the one on the plausible absence of deleterious synergistic or additive effects must imperatively be validated by complementary studies. Besides, our conclusions are only applicable to the present OPI amounts in use and the consequences of a greater pesticide consumption on várzeas remain unknown.

To further address the issues raised by this study we strongly encourage local scientific authorities to develop a monitoring program aiming at the assessment of contamination levels of a wide array of pesticides in the ichthyofauna. Particularly, it would be of first interest to characterize the seasonal fluctuations of the contamination, considering the huge hydrological modifications taking place throughout the year and their impact on várzea’s agriculture. It would also be interesting to determine the actual effect of floods on the fate of pesticide residues in the soils of várzeas: a further degradation (hydrolysis) or a desorption toward the water column.

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