# LEVELS AND DISTRIBUTION OF ORGANOCHLORINE COMPOUNDS AND BROMINATED FLAME RETARDANTS IN FISH FROM LAOS

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**Abstract**

Organochlorines (OCs) and brominated flame retardants (BFRs) were determined in three species of fish collected from various locations in Laos to understand the levels and distribution in the environment. PCBs, OC pesticides and BFRs were detected in fish from all locations, indicating ubiquitous contamination by organohalogen compounds in the environment of Laos. PCBs and DDTs were the major contaminants detected in fish of the present study, whereas other OCs and BFRs were lower. Levels of OCs and BFRs in fish from Laos are lower when compared to those from other several countries, indicating low magnitude contamination by these compounds in Laos. Relatively high concentrations of OCs and BFRs were generally found in fish collected from areas receiving discharges from human activities and waste dumping site, indicating urban areas and waste dumpsites as major contamination sources in the environment of Laos. To our knowledge, this is the first study to report concentrations of OCs and BFRs in fish from Laos. This study provides useful baseline data for future research on environmental occurrence and bioaccumulation of these compounds in the environment of Laos.

## Introduction

For several decades, organochlorine compounds (OCs) have been found in a range of environmental media and biota, and their toxic impacts to wildlife and humans is a major issue that gives rise to concerns at local, national, regional, and global level. Although, in recent years, levels of OCs in the environment have shown decreasing trends with time due to their restriction in use, brominated flame retardants (BFRs) - a group of chemicals which are still in use in a wide variety of consumer products and have similar fate, behavior and toxic potencies with those of OCs, appear to be increasing in the environment and food chains, especially the polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs)[1,2]. Therefore, environmental contamination and bioaccumulation of these compounds are also a matter of growing concern. However, investigations regarding the environmental issues by these compounds and their fate, behavior and toxic effects have been limited mostly among developed countries.

Laos is a tropical agricultural country located in Mekong Basin (Figure 1), a region which is rich in biodiversity. As a result of the industrial, urban and agricultural development, some localized areas linked to urban activities and intensive agriculture face degradation of water quality due to discharges of pollutants including surfactants, antibiotics, pesticides and various toxic chemicals[3]; however, no comprehensive survey has been conducted on the contamination by persistent toxic pollutants in the environment of Laos. In this study, concentrations of OCs and BFRs were determined in several freshwater fish species from different locations in Laos to understand their occurrence, distribution and sources.

## Materials and Methods

*Samples and Study Area*

A total of 30 individual fish, belonging to 3 species including snakehead (*Channa sp.*), tilapia (*Oreochromis mossambicus)* and carp (*Cyprinus carpio*), which are all commercially important fish consumed by local population, were collected during 2005 from 5 different locations in Laos, namely Beung That Luang-1 (snakehead collected from location affected by municipal waste water in urban area), Beung That Luang-2 (tilapia collected from lake/lagoon in urban area), B. Phonhong (snakehead collected from rural area), B. Bo-O (tilapia, intensive aquaculture area) and Lak Sipsie (carp collected from municipal dumpsite) (Figure 1). In the field, fish were identified, and their body length and weight were recorded. All the fish samples were kept in clean polyethylene bags and transported to laboratory in boxes packed with dry ice. In the laboratory, muscle tissues of fish were individually homogenized. All the samples were stored at -20$^{o}$C in the environmental specimen bank (*es*-BANK) of Ehime University until chemical analysis[4].

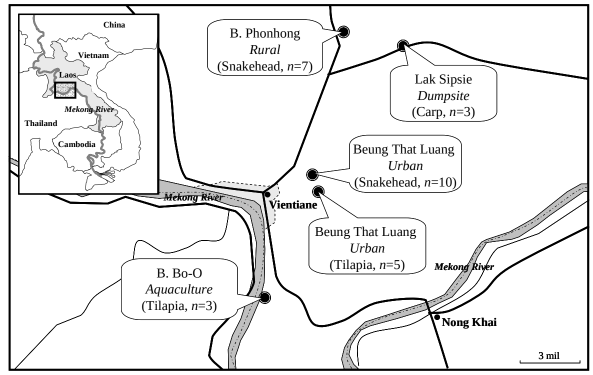


**Figure 1.** Map showing sampling locations in Laos.

*Chemical Analysis*
Analysis of OCs and BFRs were carried out according to the method described elsewhere[5], with slight modification. Following the extraction and clean-up procedures, OCs including polychlorinated biphenyls (PCBs) and OC pesticides (OCPs), such as dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexane isomers (HCHs), chlordane compounds (CHLs) and hexachlorobenzene (HCB) were determined by gas chromatography with an electron capture detector (GC-ECD). In addition, new emerging contaminants of BFRs, including PBDEs (14 PBDE congeners: BDE-3, -15, -28, -47, -99, -100, -153, -154, -183, -196, -197, -206, -207, and BDE-209) and HBCDs ($\alpha$-, $\beta$-, $\gamma$-HBCD) were quantified using gas chromatography with a mass spectrometry detector (GC-MS) and liquid chromatography with tandem mass spectrometry detector (LC-MS-MS), respectively. In this study, concentrations of OCs and BFRs are expressed as ng/g lipid wt.

*Statistical Analysis*
Statistical analysis was performed using Kruskal-Wallis one-way analysis by ranks to compare all groups. Mann-Whitney *U*-test was subsequently used to compare each group, and Spearman rank correlation was used to examine the strength of associations between parameters. All statistical analyses were performed using Stat View program version 5. A probabilistic $p<0.05$ value is considered significant.

**Results and Discussion**
*Levels and Distribution*
Concentration of OCs and BFRs measured in different fish species and locations from Laos are shown in Table 1. Among the contaminants, DDTs and PCBs were the major pollutants in almost all the samples ranging from 40-200 ng/g lipid wt. and 6.8-130 ng/g lipid wt., respectively, whereas other compounds were one to two orders of magnitude lower (DDTs>PCBs>CHLs>HCHs>HCB≈PBDEs≈HBCDs). In this study, residue levels of all contaminants did not significantly correlate with gender ($p>0.05$) (data not shown), thus data of all the male and female fish were pooled for further discussion.

Concentrations of some organohalogen compounds varied significantly among the fish species ($p<0.05$), indicating species specific accumulation and/or uptake of the compounds possibly due to their different feeding habits. For example, CHLs were found to be significantly higher in snakehead and carp than tilapia, whereas HBCDs were higher in carp than in snakehead and tilapia. Since each species were collected from locations with different background activities, the variations in the levels of contaminants in fish of the present study might be due to the location-specific exposure rather than species specific accumulation. For instance, except DDTs and HCB, all OCs were found to be significantly higher in fish collected from locations close to intensive human activities (Beung That Luang) than fish from rural (B. Phonhong) and aquaculture areas (B. Bo-O). In this study, farmed fish from B. Bo-O generally had the lowest levels of PCBs, CHLs and PBDEs, indicating low uptake of these compounds from commercial fish feed. Interestingly, significantly higher concentrations of CHLs, PBDEs and HBCDs were found in fish from Lak Sipsie (close to waste dumpsite) than other locations, indicating municipal dumpsites are important sources for these compounds to the surrounding areas.

Table 1. Concentrations of organochlorines and brominated flame retardants (ng/g lipid wt.) in fish from Laos.

| Compound | **Beung That Luang (Snakehead, *n*=10)** | | **B. Phonhong (Snakehead, *n*=5)** | | **B. Bo-O (Tilapia, *n*=5)** | | **Beung That Luang (Tilapia, *n*=7)** | | **Laksipsie (Carp, *n*=3)** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean±SD** | **Range** | **Mean±SD** | **Range** | **Mean±SD** | **Range** | **Mean±SD** | **Range** | **Mean±SD** | **Range** |
| **Lipid (%)** | 0.45±0.31 | 0.14-1.0 | 0.52±0.15 | 0.30-0.65 | 5.2±1.3 | 3.8-6.7 | 0.54±0.36 | 0.26-1.2 | 1.8±1.6 | 0.69-3.7 |
| **ΣPCBs** | 64±38 | 10-130 | 37±13 | 19-54 | 11±3.4 | 6.8-16 | 84±29 | 37-120 | 100±95 | 27-210 |
| *p,p'*-DDE | 78±34 | 28-120 | 42±16 | 21-60 | 17±1.8 | 13-18 | 42±14 | 21-63 | 32±25 | 16-60 |
| *p,p'*-DDD | 13±19 | <0.061-53 | 8.4±8.5 | 3.2-19 | 13±2.4 | 10-17 | 20±4.9 | 13-26 | 10±12 | 3.3-24 |
| *p,p'*-DDT | 16±14 | 2.7-42 | 5.4±2.9 | 2.5-10 | 24±4.0 | 18-29 | 2.5±1.3 | 1.3-5.3 | 1.0±0.25 | 0.82-1.3 |
| **ΣDDTs** | 110±57 | 40-200 | 57±13 | 45-75 | 54±7.9 | 42-64 | 65±19 | 35-89 | 86±20 | 20-86 |
| α-HCH | 0.050±0.16 | <0.045-0.49 | <0.045 | <0.045 | 0.17±0.032 | 0.14-0.22 | nd | <0.045 | 0.080±0.14 | <0.045-0.24 |
| β-HCH | 4.6±6.5 | 0.80-21 | 0.85±0.43 | 0.46-1.5 | 0.73±0.055 | 0.67-0.79 | 2.4±1.4 | 1.1-5.4 | 0.95±0.15 | 0.85-1.1 |
| γ-HCH | 0.33±0.70 | <0.039-2.1 | 0.11±0.17 | <0.039-0.38 | 0.20±0.023 | 0.18-0.24 | nd | <0.039 | 0.21±0.24 | 0.039-0.48 |
| **ΣHCHs** | 5.0±7.2 | 1.1-23 | 0.96±0.60 | 0.46-1.9 | 1.1±0.073 | 1.0-1.2 | 2.4±1.4 | 1.1-5.4 | 1.2±0.12 | 1.1-1.4 |
| *oxy*-CA | 2.0±1.6 | 0.77-5.8 | 0.49±0.19 | 0.23-0.66 | 0.060±0.058 | <0.069-0.13 | 0.08±0.14 | <0.069-0.31 | 12±19 | 0.61-34 |
| *trans*-CA | 1.3±3.1 | <0.089-9.5 | 1.3±2.9 | <0.089-6.6 | 0.30±0.053 | 0.24-0.38 | nd | <0.089 | 270±430 | 1.6-760 |
| *cis*-CA | 2.5±3.0 | <0.10-9.9 | 0.80±0.35 | 0.45-1.2 | 0.68±0.10 | 0.56-0.84 | 0.51±0.69 | <0.10-1.7 | 260±420 | 1.7-740 |
| *trans*-nona | 5.8±4.3 | 1.5-16 | 1.3±0.42 | 0.72-1.7 | 0.58±0.15 | 0.36-0.72 | 1.7±1.2 | 0.22-3.3 | 210±340 | 1.7-740 |
| *cis*-nona | 2.9±1.6 | 0.54-5.4 | 0.51±0.35 | 0.026-0.97 | 0.36±0.047 | 0.30-0.42 | 1.2±0.16 | 1.0-1.5 | 36±56 | 0.40-200 |
| **ΣCHLs** | 14±13 | 2.8-47 | 4.3±2.6 | 2.1-8.7 | 2.0±0.31 | 1.7-2.5 | 3.5±1.8 | 1.3-5.6 | 790±1200 | 5.8-2200 |
| **HCB** | 1.8±0.86 | 1.1-40 | 2.3±0.69 | 1.3-3.2 | 1.4±0.71 | 0.89-2.7 | 1.8±1.8 | 0.49-5.3 | 0.95±0.15 | 0.49-5.31 |
| BDE-3 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 | <0.028 |
| BDE-15 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 | <0.051 |
| BDE-28 | 0.31±0.0.094 | <0.025-0.28 | 0.83±0.065 | <0.025-0.17 | 0.030±0.007 | 0.025-0.041 | 0.18±0.052 | 0.13-0.26 | 0.46±0.58 | 0.074-1.1 |
| BDE-47 | 0.77±0.65 | 0.17-2.3 | 0.29±0.20 | 0.17-0.65 | 0.57±0.046 | 0.49-0.61 | 1.4±0.73 | 0.74-2.4 | 2.7±2.0 | 0.83-4.8 |
| BDE-99 | 0.34±0.38 | <0.031-0.99 | 0.23±0.16 | <0.031-0.42 | 0.059±0.017 | 0.033-0.073 | 0.12±0.19 | <0.031-0.46 | 0.21±0.18 | 0.041-0.40 |
| BDE-100 | 0.35±0.83 | <0.052-2.6 | 0.18±0.066 | 0.11-0.27 | 0.099±0.011 | 0.081-0.11 | 0.20±0.17 | <0.052-0.42 | 0.42±0.31 | 0.13-0.76 |
| BDE-153 | 0.17±0.45 | <0.041-1.4 | <0.041 | <0.041 | 0.030±0.007 | 0.035-0.066 | <0.041 | <0.041 | 0.49±0.36 | 0.13-0.85 |
| BDE-154 | 0.18±0.43 | <0.051-1.3 | 0.12±0.20 | <0.01-0.46 | 0.57±0.046 | 0.062-0.092 | 0.080±0.13 | <0.01-0.26 | 0.64±0.52 | 0.17-1.2 |
| BDE-183 | 0.17±0.36 | <0.045-1.0 | <0.045 | <0.045 | <0.045 | <0.045 | <0.045 | <0.045 | 0.24±0.38 | <0.01-0.68 |
| BDE-196 | 0.077±0.22 | <0.036-0.69 | 0.021±0.046 | <0.036-0.10 | <0.036 | <0.036 | <0.036 | <0.036 | <0.036 | <0.036 |
| BDE-197 | <0.048 | <0.048 | 0.020±0.045 | <0.048-0.10 | <0.048 | <0.048 | <0.048 | <0.048 | <0.048 | <0.048 |
| BDE-206 | 0.017±0.050 | <0.040-0.15 | <0.040 | <0.040 | <0.040 | <0.040 | <0.040 | <0.040 | <0.040 | <0.040 |
| BDE-207 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 | <0.13 |
| BDE-209 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 | <0.57 |
| **ΣPBDEs** | 2.1±2.3 | 0.70-8.2 | 0.94±0.59 | 0.40-1.9 | 0.88±0.090 | 0.73-0.95 | 2.0±0.91 | 0.93-3.2 | 5.1±4.3 | 1.4-9.8 |
| α-HBCD | 0.28±0.65 | <0.020-1.9 | <0.020 | <0.020 | 0.27±0.042 | 0.20-0.31 | 0.17±0.40 | <0.020-1.0 | 5.4±2.4 | 2.7-7.2 |
| β-HBCD | <0.020 | <0.020 | <0.020 | <0.020 | 0.051±0.034 | <0.020-0.089 | 0.074±0.18 | <0.020-0.45 | <0.020 | <0.020 |
| γ-HBCD | 0.16±0.31 | <0.020-0.94 | 0.24±0.33 | <0.020-0.64 | 0.047±0.010 | 0.035-0.060 | 0.33±0.32 | <0.020-0.87 | 1.6±2.7 | <0.020-4.8 |
| **ΣHBCDs** | 0.45±0.94 | <0.020-2.9 | 0.24±0.33 | <0.020-0.64 | 0.37±0.072 | 0.27-0.44 | 0.57±0.68 | <0.020-1.8 | 7.0±4.6 | 2.9-12 |

SD=standard deviation, na=not available (not yet analyzed).

Levels of DDTs were relatively uniform among the locations, indicating ubiquitous usage of this pesticide in Laos. In almost all fish species from all the locations, only low percentage of *p,p'*-DDT could be measured, but the profiles of DDTs in fish collected from aquaculture area close to Mekong River (B. Bo-O) showed higher proportion of the parent compound, *p,p'*-DDT (Table 1). This result indicates recent usage of DDT in and around Mekong River. This difference in the profile may also be due to difference in feeding preferences for farmed fish.

Levels of OCs in fish from Laos corresponded to the levels observed in various fish species from other parts of Mekong Basin[5-7]. For example, catfish sampled from dumpsite and aquaculture areas in Mekong Delta (South Vietnam) were found to contain 7.2-50 ng/g lipid wt. of PCBs and 59-390 ng/g lipid wt. of DDTs[5]. Various freshwater fish collected from Cambodia had on average 16 ng/g lipid wt. of PCBs and 420 ng/g lipid wt. of DDTs[6].

In Thailand, concentrations of PCBs and DDTs in freshwater fish were on average 30 ng/g lipid wt. and 120 ng/g lipid wt., respectively[7]. The levels, however, are still lower than those in fish from other Asian countries such as India[7], Indonesia[8] and China[9].

Similarly, levels of PBDEs and HBCDs in fish from Laos were also very low, ranging from 0.70-9.8 ng/g lipid wt. and <0.020-12 ng/g lipid wt., respectively. HBCDs were not present in 32% of the samples. Such low levels are probably related to a limited usage of BFRs in Laos. BDE-47 showed the highest levels, while PBDE congeners with five or more bromine atoms accumulated to a lesser extent. Deca-BDE congener was not detected in fish of the present study. The levels of BFRs observed in fish from Laos were comparable with those in farmed- and dumpsite-catfish from South Vietnam (Mekong Delta)[5]. The levels were lower than those measured in the same species as well as other marine and freshwater fish from other Asian countries such as Indonesia[10] and Taiwan[11].

*Accumulation According to Body-Length*

Significant positive correlation between fish length and concentration of DDTs was observed (r=0.77, $p<0.001$), but not for PCBs (Figure 2) and other compounds (data not shown). This result may suggest that the accumulation of DDTs in fish has not reached a steady state due to their abundance and continuous input into the environment of Laos, and thus fish bioaccumulate and biomagnify these compounds throughout their life. Unlike DDTs, it is plausible that the accumulation of PCBs, CHLs, HCHs, HCB, PBDEs and HBCDs has reached a steady state, indicating that the elimination or metabolic rates are enough to compensate dietary intake. In fact, most of these compounds were found to be relatively low in fish of the present study.

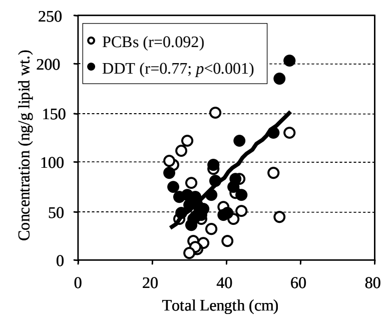


Figure 2. Relationship between fish length and concentrations of PCBs and DDTs.

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