

# Semi Aquatic Top-Predators as Sentinels of Diversity and Dynamics of Pesticides in Aquatic Food Webs: The Case of Eurasian Otter (*Lutra lutra*) and Osprey (*Pandion haliaetus*) in Loire River Catchment, France

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## 1. Introduction

The Eurasian otter (*Lutra lutra*, *Lutrinae*, figure 1) and the osprey (*Pandion haliaetus*, *Pandionidae*, figure 2), formerly widespread in Europe and in France, have strongly declined during the 20th Century, following direct persecutions, habitat alteration and pollution, and consecutively decline of main prey. Direct persecutions were perpetrated because both species were considered as active competitors for fishing activity: otters were massively trapped for fur, and osprey populations were dismantled by direct shot or egg destruction in nests. Otter and osprey are both semi-aquatic top-predators species: diet is highly dominated by fish, which constitute at least 80 % and almost 98-100 % of the averaged prey biomass consumed by otter and osprey, respectively. However, diet studies of otter and osprey never showed any strong predation impact on fish diversity or biomass in rivers (reviews in Poole, 1989; Thibault et al. 2001; Clavero et al. 2003; Britton et al. 2005; Kruuk, 2006; Dennis, 2008). This diet specificity influence otter physiological characteristics: by comparison with other mammalian carnivores, a specific diversity and accumulation pattern of essential fatty acids of aquatic origin from food to otter tissues was recently shown (Koussoroplis et al. 2008). Another diet characteristic of both species is the diversity of prey and their opportunistic hunting behaviour: almost all fish species available in otter or osprey local habitat are able to be consumed, depending on hunting conditions, with important diet variations between seasons, during life cycle or between populations. This is particularly true concerning osprey, a migrating species present in northern and western Europe during reproductive season, and wintering from southwestern Europe to sub-Saharan Africa, but was also observed concerning sedentary otter. Because of their high trophic level, habitat requirements and main ecological characteristics, otter and osprey can be considered as good sentinels and indicator species of global contamination and biomagnification of toxic contaminants in aquatic food webs of large rivers, estuaries, reservoirs and lakes.



Fig. 1. Eurasian Otter (*Lutra lutra*, adult male, photo R. Rosoux).

Indeed, after direct destructions, contamination by persistent pollutants (*e.g.* pesticides, polychlorinated biphenyls (PCBs) or heavy metals) is blamed to be the causative agent of the decline of otter and osprey populations, throughout Europe as elsewhere in the world. On seldom occasions, *acute poisoning* after direct exposure or secondary poisoning by ingestion of highly contaminated prey were observed on otters in France, especially after oil spills, other industrial accidents or following heavy treatments with insecticides, avicides or rodenticides (Fournier-Chambrillon et al. 2004; Lafontaine et al. 2005; Berny, 2007; Lemarchand et al. 2010). Elsewhere in the world, acute poisoning of many marine otters (*Enhydra lutris*) were reported after Exxon Valdez oil spill in 1989 in Alaska (Garshelis and Jonhson, 2001). On the other side cases of acute poisoning were very rarely observed on ospreys. Potential long-term effects of trophic originating toxic compounds (*i.e. chronic poisoning*) on otters and ospreys were often investigated through reports of contamination or intoxication cases on monitored populations. Pesticides, and particularly organochlorine (OC) pesticides were the most commonly analyzed elements (but also heavy metals and PCBs, these latter generally associated with OC pesticides to assess the total OC contamination). Pesticides uses in the European Union are clearly specified by Directive EC 91/414, but illegal poisoning of wild, game and domestic animals still occurs, and often results from pesticides abuse or illegal use (Berny, 2007; Berny and Gaillet, 2008). Among OC pesticides, dichlorodiphenyltrichloroethane (DDT) and its main metabolites, particularly dichlorodiphenyldichloroethylene (DDE), were shown to accumulate in otter and osprey, causing body condition alteration, direct reproductive failure (like eggshell thinning observed on osprey) and consecutively population decline (Spitzer et al. 1978; Wiemeyer et al. 1988; Mason and Macdonald 1993a, b; Ewins et al. 1999; Elliott et al. 2000; Ruiz-Olmo et al. 2000; Henny et al. 2008). As these deleterious effects were documented on a very large spectrum of wild and domestic species after insect pests control, DDT uses were severely controlled or banned from the 1970's in developed countries (ban from 1973 in France). Nevertheless, DDT is still used in developing countries, particularly in India or Africa by indoor spraying during anti-malaria campaigns. The risk of DDT flow into agricultural and

aquatic systems or wild environment of these countries was recently underlined (UNEP, 2008). Due to environmental stability and persistence of DDT and metabolites, and probably following post-ban use of old stocks, these compounds are still present in environment and were recently detected in otters or ospreys (Kannan et al. 2004; Lemarchand et al. 2007, 2010; Henny et al. 2008). For this latter species, continuing of DDT use in some developing countries is an additional threat during the wintering period. Lindane, and to a lesser extent, Aldrin, Endosulfan and Methoxychlor were the main other OC pesticides quantified in otter or osprey during previous studies. However, most of studies reported low concentrations of these compounds when compared to DDT residues, and therefore a limited contribution to total OC contamination (Wiemeyer et al. 1988; Mason and Macdonald, 1993a,b, 1994; Elliott et al. 2000; Rattner et al. 2004; Lemarchand et al. 2010).

Cholinesterase inhibitors as organophosphate (OP) and carbamate (CA) pesticides were widely used worldwide as insecticides for the protection of cultivated plants or livestock. Direct and indirect toxicity of cholinesterase inhibitors (e.g. Carbofuran, Mevinphos) were underlined on insect-consumer birds, birds of prey and scavengers like white-tailed sea eagle (*Haliaeetus albicilla*) or red kite (*Milvus milvus*) (Hart et al. 1993; Elliot et al. 1996; Berny and Gaillet, 2008). These insecticides are highly toxic to birds of prey, nevertheless intoxication cases following OP and CA pesticides contamination remained rare when compared to total reported deaths (Fleischli et al. 2004). Pyrethroids insecticides were recently preferred to OP and CA pesticides uses. Indeed, pyrethroids pesticides are considered as a safer method of pest control because of their lower direct toxicity on mammals and birds (Martin et al. 1998; Chu et al. 2005). Nevertheless, data on pyrethroids insecticides diversity, persistence or toxicity in wild fauna are very poor in literature concerning top predators species.



Fig. 2. Osprey (*Pandion haliaetus*, adult female; photo C. Lemarchand)

Related to their plant-specific metabolic action, water solubility and poorly lipophilic characters, herbicides are documented as less toxic pesticides to vertebrates than insecticides

(Berny, 2007). Data on herbicides diversity and toxicity on vertebrates or predators, especially otters or ospreys, are particularly rare. Nevertheless, some studies demonstrated a direct effect of herbicides on herbivorous mammals or bird diversity or abundance during land use modifications (Santillo et al. 1989a,b). Bioaccumulation potential of herbicides to a top predator was recently confirmed by a study in Washington State (USA) on sediments, fish and ospreys (Chu et al. 2007). Furthermore, recent studies underlined a direct impact of herbicides, particularly triazines, on fish and amphibians' reproduction or survival (Langlois et al. 2009; Tillitt et al. 2010). Direct impact of herbicides on fish or amphibians' populations would indirectly affect otters and ospreys by a reduction in food resource. Therefore toxicity of some persistent herbicides on vertebrates could be underestimated by an insufficient risk evaluation. At the beginning of the 1980's in France, otters only survived in two distinct populations: in the Massif Central mountains (centre), and along Atlantic Ocean and western wetlands of the country (Bouchardy, 1986). At the same period, osprey had disappeared of continental France as a nesting species. Legal protection of the otter and the osprey was decided from 1976. First signs of species recovery or return were recorded soon after. From 1985 increase and expanding of otter populations were proved and monitored in the whole repartition area of the species in France (Bouchardy, 1986; Rosoux and Bouchardy, 2002). In spring 1984, one pair of osprey stopped its migration towards northern Europe and built a nest along Loire River. Species is nesting again in continental France since 1985 (Coll., 1996). As osprey is a semi-colonial and philopatric species, other pairs quickly mated close to the first one, starting a new expanding population. European directives and national action plans allowed the protection and / or the restoration of both species habitat (Rosoux et al, 1999; Nadal and Tariel, 2008; Kuhn, 2009). The main characteristic of these species recoveries is their entirely natural process. Indeed, otter and osprey were never been reintroduced or reinforced in France, in order to establish habitats requirements, main natural and anthropogenic limits to populations, to locate colonization corridors and major sites for reproduction and breeding. After about three decades of protection, otter population in France is still increasing, formerly isolated populations met from the beginning of the 2000's and the repartition area of the species covers the whole Massif Central related to the western third of the country (Bouchardy et al. 2001; Kuhn, 2009; Lemarchand and Bouchardy 2011). 37 reproductive pairs of ospreys were noted in 2010 in continental France, mainly distributed along the medium part of the Loire River, but a geographical expansion of the species towards other river systems was recently noted (Nadal and Tariel, 2008). Increase of otter and osprey populations particularly concerns Loire River catchment, a major dispersal corridor that should be decisive for species conservation and dispersion in the whole country. As many predators, otter and osprey suffered from a bad reputation, but are now associated with preserved habitats and food resource (Chanin, 2003; Whitfield et al. 2003; Grove et al. 2009). However, otter and osprey remain sparse in France and are listed on UICN National Red Lists as "Minor preoccupation (LC)" and "Vulnerable (VU)", respectively (UICN France et al. 2008, 2009). Objectives of this study were to evaluate the contamination of two flagship species (European otter and osprey) by a wide spectrum of pesticides, using a standard protocol of pesticides analyses in wild or domestic fauna and a non-invasive animal approach during a natural recolonization process in Loire River catchment. Since 2004 for the otter and 2007 for the osprey, a large toxicological program was launched during the "Plan Loire Grandeur Nature" program in France. 45 pesticides, including herbicides, organochlorine, organophosphate, carbamate and pyrethroids pesticides and a few main metabolites were systematically analyzed in otters and ospreys (but also in great cormorants, freshwater fish and invertebrates) from Loire River catchment (Lemarchand et al. 2007, 2009, 2010).

## 2. Materials and methods

### 2.1 Study area

The study area corresponded to the whole Loire River and main tributaries catchment in France (Fig. 3). Loire River catchment (117000 km<sup>2</sup>, total length of rivers and tributaries of about 40000 km) is characterized by an important diversity of habitats and species, and is considered as one of the most preserved large hydrosystems in Western Europe. A national and European action plan, "Plan Loire Grandeur Nature", is running since 1994 to study and conserve this diversity, but also to protect inhabitants from floods and to maintain economic activity.



Fig. 3. Map of the Loire River (bold) catchment in mainland France

### 2.2 Animals monitoring and sampling

Concerning such rare species, it is particularly difficult to obtain sufficient sample material from enough individuals to support analysis and statistics. For ethical reasons it was not imaginable to trap or kill otters or ospreys for analyses. To avoid any vital risk related to handling, capture and bleed of animals were not considered. Furthermore, otter and osprey are fully protected by national and international laws, and listed as species of interest by the European Community (Habitats Directive 92/43/EC, Birds Directive 79/409/CEE). All operations were therefore entirely conducted under appropriate authorizations by a non-invasive approach. A large network, constituted by people in charge of otter and osprey studying and monitoring in mainland France was built to organize and enhance sampling under the coordination of the Muséum d'Orléans. The national agency for game and wildlife (ONCFS), hunting federations (FDC), the national agency for water and aquatic environments (ONEMA), health centres of the national union (UFCV) and of the birds protection league (LPO - French representative of Bird Life International), the national research centre on birds population biology (CRBPO, associated with the French national museum of natural history MNHN and Mr Rolf Wahl, in charge of osprey ringing program in France), the French Ministry of Environment (MEEDDM and DREAL Centre), the national agency for forests (ONF), private land owners and companies, museums, associations ("Loiret Nature Environnement") and regional naturalists were contributors for this study.

Concerning otters, only road-traffic killed individuals and those found dead in the wild in study area were collected. Based on visual observation, carcasses found more than 24h (during summer) or 48h (during winter) after road collision were considered too degraded

and not taken into account for post-mortem examination and toxicological analyses. Concerning ospreys, non-hatched eggs and dead young in nests were collected during chicks ringing operations. As scientists and birdwatchers monitor a majority of osprey nests in continental France, non-hatched eggs and dead young in nests were reported and sampled as soon as possible. France is also a major crossing area for migrating osprey from different populations (Hake et al. 2001; Dennis, 2008; Strandberg et al. 2009). Due, in one way, to the extreme rarity of this species in continental France (less than one hundred reproductive individuals), and in an other way that “foreigners” individuals (*i.e.* born in neighbour countries, but potentially breeders in France) are able to be found dead within the national territory (naturally or after illegal shots, electrocution on power cables, or drown in fish farms), migrating individuals flying towards reproduction areas elsewhere in Europe (Germany, Great-Britain, Scandinavia) completed sampling.

All samples were deep-frozen (-40 °C) prior to analyses. For each otter or osprey carcass, a necropsy was performed, and about 20 g of liver was sampled. This organ was preferred to fat because some otters and a lot of ospreys have very little fat, particularly at the end of spring migration concerning these latter. Otter sex and weight were determined; animals were measured (total and head, body, foot and tail lengths). Age was defined as “juvenile” (milk teeth, little size and weight), “subadult” (adult size and weight, teeth without wear and tartar) and “adult” or “old” (worn teeth with tartar). Body condition index  $K$  was determined according to Kruuk and Conroy (1991). Osprey sex and weight were determined; animals were measured (wing, body, foot and tail lengths). Non-hatched osprey eggs were drilled and emptied; eggshell was conserved for future studies on shell thickness. Age was defined as “egg” (non-hatched), “juvenile” (non-flying hatched individual), “subadult” (emancipated individual with the characteristic creamy fringe on feathers) and “adult” (adult size and plumage) (Dennis, 2008). Each animal (otter or osprey) is characterised by a specific case-record gathering discovery circumstances, clinical and biometrical data. After necropsies, carcasses were conserved for further showing or collection in museums or, if too degraded, systematically destroyed according to law.

### 2.3 Choice of compounds

Pesticides uses in France are one of the biggest in the world. Various compounds have been used for wood, vineyards, orchard, crops or ornamental plant protection, human or livestock health, and roads, railways or boat maintenance. Origins and flow of compounds are complex and aquatic habitats are exposed to both direct and indirect contamination.

In such a generalist approach, the choice of analyzed compounds is crucial and has to be representative:

- Of the diversity of uses (agrochemicals, industrials or domestics) in study area,
- Of the available analytical techniques and limits,
- Of accumulation and transfer patterns from trophic webs components to studied species.

A specific detection and quantification methodology was developed for pesticides in the toxicology laboratory of the college of veterinary medicine (VetAgro Sup, Lyon, France) during routine analyses on wild, game or domestic fauna. Compounds were chosen according to their toxicity on fauna, persistence in soil and water and accumulation in food webs. Regular complements and upgrades were added, as a function of new compounds or new detection techniques. Detected compounds are listed in Table 1 below.

Pesticides	Pesticides family and main use	Molecular formula	Date of ban in France (or current status)
Lindane ( <i>gamma</i> -HCH)	Organochlorine insecticide	C <sub>6</sub> H <sub>6</sub> Cl <sub>6</sub>	1998
Endosulfan	Organochlorine insecticide	C <sub>9</sub> H <sub>6</sub> Cl <sub>6</sub> O <sub>5</sub> S	2007
DDT	Organochlorine insecticide	C <sub>14</sub> H <sub>9</sub> Cl <sub>5</sub>	1972
Heptachlor	Organochlorine insecticide	C <sub>10</sub> H <sub>5</sub> Cl <sub>7</sub>	1973
Aldrin	Organochlorine insecticide	C <sub>12</sub> H <sub>8</sub> Cl <sub>6</sub>	1992
Methoxychlor	Organochlorine insecticide	C <sub>16</sub> H <sub>15</sub> Cl <sub>3</sub> O <sub>2</sub>	2002
Methiocarb	Carbamate insecticide, molluscicide	C <sub>11</sub> H <sub>15</sub> NO <sub>2</sub> S	Still in use
Carbofuran	Carbamate insecticide	C <sub>12</sub> H <sub>15</sub> NO <sub>3</sub>	2008
Mevinphos	Organophosphate insecticide	C <sub>7</sub> H <sub>13</sub> O <sub>6</sub> P	2004
Phorate	Organophosphate insecticide	C <sub>7</sub> H <sub>17</sub> O <sub>2</sub> PS <sub>3</sub>	2004
Dichlorvos	Organophosphate insecticide	C <sub>4</sub> H <sub>7</sub> Cl <sub>2</sub> O <sub>4</sub> P	2007
Terbufos	Organophosphate insecticide	C <sub>9</sub> H <sub>21</sub> O <sub>2</sub> PS <sub>3</sub>	2004
Diazinon	Organophosphate insecticide	C <sub>12</sub> H <sub>21</sub> N <sub>2</sub> O <sub>3</sub> PS	Still in use
Disulfoton sulfone	Organophosphate insecticide	C <sub>8</sub> H <sub>19</sub> O <sub>2</sub> PS <sub>3</sub>	2004
Chlorpyrifos ethyl	Organophosphate insecticide	C <sub>9</sub> H <sub>11</sub> Cl <sub>3</sub> NO <sub>3</sub> PS	Still in use
Fenitrothion	Organophosphate insecticide	C <sub>9</sub> H <sub>12</sub> NO <sub>3</sub> PS	Still in use
Pyrimiphos methyl	Organophosphate insecticide	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>3</sub> PS	Still in use
Malathion	Organophosphate insecticide	C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub>	2008
Fenthion	Organophosphate insecticide	C <sub>10</sub> H <sub>15</sub> O <sub>3</sub> PS <sub>2</sub>	2005
Parathion	Organophosphate insecticide	C <sub>10</sub> H <sub>14</sub> NO <sub>3</sub> PS	2002
Methidathion	Organophosphate insecticide	C <sub>6</sub> H <sub>11</sub> N <sub>2</sub> O <sub>4</sub> PS <sub>3</sub>	2004
Triazophos	Organophosphate insecticide	C <sub>12</sub> H <sub>16</sub> N <sub>3</sub> O <sub>3</sub> PS	1992
Trifluraline	Anilide herbicide	C <sub>13</sub> H <sub>16</sub> F <sub>3</sub> N <sub>3</sub> O <sub>4</sub>	2008
Atrazine	Triazine herbicide	C <sub>8</sub> H <sub>14</sub> ClN <sub>5</sub>	2003
Simazine	Triazine herbicide	C <sub>7</sub> H <sub>12</sub> ClN <sub>5</sub>	2003
Terbutylazine	Triazine herbicide	C <sub>9</sub> H <sub>16</sub> ClN <sub>5</sub>	2003
Cyanazine	Triazine herbicide	C <sub>9</sub> H <sub>13</sub> ClN <sub>6</sub>	2004
Alachlor	Chloroacetanilide herbicide	C <sub>14</sub> H <sub>20</sub> ClNO <sub>2</sub>	2008
Metolachlor	Organochlorine herbicide	C <sub>15</sub> H <sub>33</sub> ClNO <sub>2</sub>	2003
Diuron	Substituted phenylurea herbicide	C <sub>9</sub> H <sub>10</sub> Cl <sub>2</sub> N <sub>2</sub> O	2008
Epoxyconazole	Fongicide	C <sub>17</sub> H <sub>13</sub> ClFN <sub>3</sub> O	Still in use
Tefluthrine	Pyrethroid insecticide	C <sub>17</sub> H <sub>14</sub> ClF <sub>7</sub> O <sub>2</sub>	Still in use
Cyhalothrine Lambda	Pyrethroid insecticide	C <sub>23</sub> H <sub>19</sub> ClF <sub>3</sub> NO <sub>3</sub>	Still in use
Permethrine Cis	Pyrethroid insecticide	C <sub>21</sub> H <sub>20</sub> Cl <sub>2</sub> O <sub>3</sub>	Still in use
Cyfluthrine 2	Pyrethroid insecticide	C <sub>22</sub> H <sub>18</sub> Cl <sub>2</sub> FNO <sub>3</sub>	Still in use
Cypermethrine 2	Pyrethroid insecticide	C <sub>22</sub> H <sub>19</sub> Cl <sub>2</sub> NO <sub>3</sub>	Still in use
Fenvalerate Cis	Pyrethroid insecticide	C <sub>25</sub> H <sub>22</sub> ClNO <sub>3</sub>	Still in use
Deltamethrine	Pyrethroid insecticide	C <sub>22</sub> H <sub>19</sub> Br <sub>2</sub> NO <sub>3</sub>	Still in use

Table 1. List of families and uses, molecular formulae, current status of the compounds analyzed in this study.

## 2.4 Pesticides quantification methods

### 2.4.1 Organochlorine pesticides

2.0-8.0 g of tissue were sampled and 30 ml of hexane/acetone 75/25 mix was added. Each sample was blended twice with an Ultraturrax® (Ika, Werke, Germany) and filtered through a phase separator membrane. The extract was evaporated at 60 °C in a rotary evaporator. The dry extract was dissolved in 10 ml hexane.

Two ml of fuming sulphuric acid (SO<sub>3</sub> 7%) were added, and after centrifugation at 4x g, 1 ml of the supernatant was used for OC pesticides quantification by gas chromatography with electron capture detection material. Temperature program and injection conditions are described in Lemarchand et al. (2007; 2010). Each sample was run in duplicate. Organochlorine pesticides concentrations were calculated by using different mix standards. Recovery level on standard mixtures was always greater than 92%. All standards were purchased from CIL (St Foy la Grande, France), and purity was > 99%. Linearity was determined between 5 and 100 ng.g<sup>-1</sup> ( $r^2 > 0.99$  on standards and spiked samples, 5-point calibration curves). Limits of detection were between 0.5 and 1.0 ng.g<sup>-1</sup> lipids for individual PCB congeners. Cod liver oil (BCR349) certified material was used as a regular quality control.

### 2.4.2 Organophosphate pesticides analyses

5 g of muscle sample was shaken with 60 ml dichloromethane and 10 g anhydrous sulfate. Mix was then filtered through a Whatman 1 PS membrane, and evaporated under vacuum at 40°C. Dry samples were diluted in 3 ml ethanol, and underwent an ultrasonic step. Extract was then purified with a Sep pack R 300 (Silica Waters, 020810; 500 mg) column conditioned with 2 ml methanol and 2 ml ethanol. 2 ml dichloromethane were used for column elution. Purified samples were dried and diluted with 3 ml dichloromethane. Organophosphate (OP) and 2 carbamates (CA) pesticides (Dichlorvos, Carbofuran, Mevinphos, Phorate, Phorate oxon, Phorate sulfone, Methiocarbe, Terbufos, Diazinon, Disulfoton, Chlorpyrifos methyl, Chlorpyrifos ethyl, Fenitrothion, Pyrimiphos methyl, Malathion, Fenthion, Parathion, Methidathion, Disulfoton sulfone, Triazophos) concentrations were determined by GC/MS in SIM mode (OP + carbofuran and methiocarbe). A 5973N MS coupled with a 6890 GC (Agilent®) was used, with a 30m HP5-MS column (0.25 mm ID, 0.25µm thickness). For each sample standard and spiked sample, 2 µL were injected. The temperature program was 100°C (2 min), 55°C/min up to 200 °C (held for 5 min), 50 °C up to 220 °C (held for 3 minutes), followed by 60 °C/min up to 300°C. A final, post-run time of 2 min at 300°C was maintained. Total run time was 13.55 min. Injector was set at 250°C and the He flow was set at 2.5 ml/min. Each OP or CA was identified based on the following criteria: retention time and 3-4 fragmentation ions with pre-defined relative amounts and 20% variability acceptance for each ion. Linearity was confirmed between 25 and 500 ng.g<sup>-1</sup> with 5 point calibration curves and  $r^2 > 0.99$ . Recovery was determined between 76% and 104% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%.

### 2.4.3 Pyrethroids pesticides analyses

5 g of tissue (liver or muscle) sample was shaken in 60 ml ethanol and 10 g anhydrous sulfate, and then filtered through a Whatman 1 PS membrane. Extract was dissolved in 5 ml methanol and underwent a second filtration procedure. Concentrations were determined by GC / ECD and confirmed by GC/MS according to a modified method of the French Food Safety Authority (Anses Met AFSSA). An Agilent GC-ECD 6850 with a 30m HP1 column



(0.32 mm ID, 0.25 $\mu$ m film) was used. For each samples standard and spiked sample, 2  $\mu$ L were injected. The temperature program was common to OCS', PCBs and pyrethroids (initial temp: 100°C, first ramp 6°C/min up to 220 °C held for 10 min, 2<sup>nd</sup> ramp 7 °C/min up to 285°C, held for 1 min, total run time 42.29 min) Injector was at 230°C, detector at 300°C. Total He flow was 9 ml/min. Pyrethroids were identified according to their retention times. Linearity was confirmed between 10 and 100 ng.g<sup>-1</sup> with 5 point calibration curves and  $r^2 > 0.99$ . Recovery was determined between 82% and 94% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%. For all positive samples, a confirmatory analysis was performed with GC/MS in SIM mode. Identification was based on retention times and 3 or 4 ions.

#### 2.4.4 Herbicides analyses

2 g of muscle sample was shaken during 5 minutes in 8 ml acetone, and then centrifuged at 4x g; supernatant was placed in separate tubes, and this extraction was performed twice. Samples were evaporated under nitrogen, and dry extract was dissolved in 1 ml acetone/methanol (50:50) solution. Extract was then purified with a SPE C18 500 mg column conditioned with 2 ml acetone and 2 ml methanol. Column was vacuum dried and purified samples were diluted in 3 ml acetone. After drying under nitrogen, samples were diluted in 1 ml methanol. Herbicides (Trifluraline, Atrazine, Simazine, Terbutylazine, Diuron, Alachlor, Metolachlor, Cyanazine, Epoxyconazole) concentrations were determined by GC/MS spectrometry. A 5973N MS coupled with a 6890 GC (Agilent®) was used, with a 30m HP5-MS column (0.25 mm ID, 0.25 $\mu$ m thickness). For each samples standard and spiked sample, 2  $\mu$ L were injected. The temperature program was 85°C held 1 min, followed by 6°C/min up to 170°C (held for 12 min), then followed by 20°C/min up to 280°C, held for 4.33 min (total run time 37 min). Injector was at 250°C and in the splitless mode. Each herbicide was identified based on the following criteria: retention time and 3-4 fragmentation ions with pre-defined relative amounts and 20% variability acceptance for each ion. Linearity was confirmed between 100 and 500 ng.g<sup>-1</sup> with 5 point calibration curves and  $r^2 > 0.99$ . Recovery was determined between 67% and 98% for all spiked samples and repeatability was considered acceptable with coefficients of variation <15%.

#### 2.5 Calculation methods and statistical analysis

Geometric means of *p,p'*-DDE, *p,p'*-DDD and *p,p'*-DDT were added to calculate the sum of DDTs ( $\Sigma$  DDTs). Geometric means of lindane, endosulfan, DDE, DDD, DDT, heptachlor, heptachlor epoxyde, aldrin and metoxychlor were summed to provide the sum of pesticide concentrations ( $\Sigma$  Pesticides). All these were chosen by the National Veterinary School of Lyon (VetAgro Sup, France) standard protocol (Mazet et al. 2005; Lemarchand et al. 2007, 2010). The Mann-Whitney test was used to compare two independent samples, Kruskal-Wallis for *k* comparisons, Spearman correlation rank test to quantify associations between two variables. Statistics were performed using *R*. (Ihaka and Gentleman 1996).

### 3. Results

#### 3.1 General characteristics of sampled material

##### 3.1.1 Otters

Otters have been systematically collected since the beginning of the toxicological program along Loire River and tributaries catchment (2004). This program allowed an increase in the

scientific use of previously collected and stocked individuals, for toxicological analyses first, but also for genetic study of otter recolonization, diet, biometry or causes of mortality approaches (Mucci et al. 2010). Main characteristics of otters analyzed in this study are summarized in table 2.

Otter	Sex	Age	Body index K	Catchment Origin	Cause of death	Otter	Sex	Age	Body index K	Catchment Origin	Cause of death
LF 01	Female	Juvenile	1,21	Upper part	Collision	LM 123	Male	Subadult	1,07	Lower part	Collision
LM 02	Male	Adult	0,91	Upper part	Collision	LM 124	Male	Subadult	1,00	Lower part	Collision
LM 05	Male	Adult	1,08	Upper part	Collision	LM 125	Male	Adult	1,04	Lower part	Collision
LM 09	Male	Subadult	1,11	Upper part	Collision	LM 126	Male	Adult	1,04	Lower part	Collision
LM 12	Male	Subadult	0,71	Upper part	Collision	LM 127	Male	Subadult	1,10	Lower part	Collision
LM 13	Male	Adult	0,91	Upper part	Collision	LM 129	Male	Adult	1,16	Lower part	Collision
LM 14	Male	Adult	1,07	Upper part	Collision	LM 130	Male	Juvenile	1,17	Lower part	Collision
LM 16	Male	Juvenile	0,62	Upper part	Natural	LM 141	Male	Juvenile	0,85	Lower part	Collision
YL	Male	Juvenile	0,82	Upper part	Starvation	LM 144	Male	Adult	0,89	Lower part	Collision
LF 62	Female	Juvenile	0,95	Lower part	Collision	LM 148	Male	Adult	1,20	Lower part	Collision
LF 64	Female	Adult	1,01	Lower part	Collision	LM 153	Male	Adult	1,36	Lower part	Collision
LF 68	Female	Juvenile	0,64	Lower part	Collision	LM 154	Male	Subadult	1,17	Lower part	Collision
LM 71	Male	Adult	1,28	Lower part	Collision	LM 156	Male	Juvenile	1,12	Lower part	Collision
LF 72	Female	Adult	1,06	Lower part	Collision	LF 157	Female	Subadult	0,82	Lower part	Collision
LF 74	Female	Subadult	0,97	Lower part	Collision	LF 158	Female	Subadult	0,58	Lower part	Collision
LF 77	Female	Old	0,67	Lower part	Collision	LF 159	Female	Juvenile	1,03	Lower part	Collision
LF 78	Female	Adult	0,71	Lower part	Collision	LM 160	Male	Adult	1,20	Lower part	Collision
LM 83	Male	Adult	0,68	Lower part	Collision	LF 161	Female	Adult	0,95	Lower part	Collision
LF 85	Female	Subadult	0,96	Lower part	Collision	LF 162	Female	Adult	0,98	Lower part	Collision
LM 86	Male	Adult	1,39	Lower part	Collision	LF 163	Female	Adult	0,83	Lower part	Collision
LF 88	Female	Adult	1,02	Lower part	Collision	LF 164	Female	Adult	0,91	Lower part	Collision
LF 89	Female	Subadult	0,96	Lower part	Collision	LF 165	Female	Adult	0,95	Lower part	Collision
LM 90	Male	Adult	0,89	Lower part	Collision	LF 167	Female	Adult	0,92	Lower part	Collision
LF 91	Female	Adult	1,03	Lower part	Collision	LF 168	Female	Adult	1,06	Lower part	Collision
LF 93	Female	Adult	0,82	Lower part	Collision	LF 169	Female	Adult	1,05	Lower part	Collision
LM 94	Male	Adult	1,38	Lower part	Collision						

Table 2. Main characteristics and causes of death of otters in this study.

Fifty-one otters were necropsied and analyzed for this study, with 24 females (47%) and 27 males (53%). Sex-ratio of the sample was very close to the equilibrium but characterized by a slight over-representation of males. This was noted in previous studies, and was generally

attributed to the larger territory of males compared to females, with associated higher risk of vehicular collision during food or new habitat foraging, particularly in the case of natural recolonization of unknown habitats (Foster-Turley et al. 1990; Rosoux and Tournebize, 1993; Kruuk, 2006; Lemarchand, 2007). Most of dead otters (30 out of 51, i.e. 59%) were adults, 11 were subadults, 9 were juvenile and only one was an old individual. This mortality picture is different from those observed in previous studies, where most of discovered otters were juvenile or subadults, with an linear increasing in probability of death with age, considering the rareness of very old individuals in nature (Kruuk and Conroy, 1991; Kruuk, 2006; Lemarchand, 2007).

With the exception of only two individuals found in the wild during the study, all otters died after a vehicular collision. These results tend to confirm that road casualties seem to be one of the main causes of mortality of otters, but, as suggested by Kruuk and Conroy (1991), and Kruuk (2006), it is the easiest way to find dead otters, those dying of other causes in the wild having far less probability of being found. This bias of carcasses collect was difficult to overlap, because of the huge human and financial costs of systematic search on riverbanks, ponds and lakes in such a study area. Considering this bias of collect, assessing the real hierarchy of causes of mortality remains hard for such a species. Among those found in the wild, one was an adult found dead without any clinical sign or injury, the other was a very little otter, only a few days aged, died of starvation after the dead or the abandon by its mother. This finding was surprising: as young otters live all their time in den, the probabilities of discover them if they die is very low (Kruuk, 2006). With the exception of various injuries caused by road collisions, all otters were in good physical conditions, with no apparent organ damage due to intoxication, like hemorrhages, organ abnormality or wound. Body condition index was systematically comprised between 0,5 and 1,4: it can be assumed that none otter collected in this study was in poor health condition or particularly fat (index < 0,5 or > 1,4, respectively; Kruuk, 2006). Medium body condition index of all otters was 0,99, very close to the value of reference (=1; Kruuk, 2006). Differences between body condition indexes K, total length or weight of otters from upper or lower part of Loire River catchment were not significant. Post-mortem examinations of otters never showed any clinical sign of severe intoxication, like organ or tissue abnormality, secretions, hemorrhages or anemia. Lead pellets were found on two occasions in carcasses but were not a death causal agent. According to Bo Madsen et al. (2000) or Simpson et al. (2005), otters are generally few concerned by natural intoxication (e.g. botulism), viral or bacterial diseases. Individuals examined here never showed any strong disease or natural intoxication signs.

### 3.1.2 Ospreys

Osprey population in mainland France is monitored since the natural come back of the species as breeding one in 1984 (synthesised in Nadal and Tariel, 2008). From only one in 1984, population of breeding ospreys in the study area increased to 35 active nests in 2010, the overall breeding success during 1985-2006 periods was 2.0 fledglings per active nest. This value is higher than the stable population threshold (=0,8), and, associated with the recorded survival rate of adults of 0.97, suggests a very good reproduction dynamics of osprey in the study area during this period (Poole, 1989; Rattner et al. 2004; Wahl and Tariel, 2006; Dennis, 2008; Nadal and Tariel, 2008). The large, favourable and non-fully occupied potential habitats along the Loire River, associated with an important and diversified food resource were the main factors of this reproductive success. Ospreys have been

systematically collected in Mainland France from the end of 2007, when toxicological program on otter was extended to this top-predator. Main characteristics of ospreys analyzed in this study are listed in table 3.

17 osprey samples collected since 2007 in France were used. As some of the birds were ringed, or came from known nests, information about age and origin was established for 12 ospreys (70%). 7 osprey samples (3 non hatched eggs, 3 dead *pulli* in nests and one adult) came from the breeding population along Loire River. The other birds were subadults or adults collected during spring or autumn migration: 4 from Germany, 1 from Norway, and 5 non-ringed birds were from unknown origin. 3 ospreys died after electrocution on power cables, 3 after illegal shots in spite of the full protection of the species by law. Drawing of ospreys in fishponds with inadequate protection nets is another cause of osprey mortality currently emerging: 4 individuals died in the same structure during spring 2009 (March 23<sup>rd</sup>, 24<sup>th</sup>, 26<sup>th</sup> and 28<sup>th</sup>) in eastern France. To minimize this drawing risk, protection nets were recently modified in several fishponds situated along osprey migration corridors. As observed concerning otters, post-mortem examination never showed any showed any clinical sign of severe intoxication, like organ or tissue abnormality, secretions, hemorrhages or anemia. Three *pulli* from the same nest died of starvation during a long period of bad weather conditions. Three cases of feather pitching syndrome were observed, but these specimen were not collected early enough to support toxicological analyses. The rest of the examined individuals were in good physical conditions (normal size and weigh) and did not show any intoxication or disease sign.

Osprey	Sex	Age	Origin	Cause of death
bbz 4	female	adult	Germany	electrocution
Bbz 3	male	adult	Loire River	electrocution
Bbz 7	unknown	egg	Loire River	non hatched egg
Bbz 8	unknown	egg	Loire River	non hatched egg
Bbz 9-11	male	juvenile	Loire River	pullus dead in nest
Bbz 12	unknown	juvenile	Loire River	pullus dead in nest
Bbz 13	unknown	egg	Loire River	non hatched egg
Bbz 14	female	subadult	Norway	illegal shot
Bbz 17	male	subadult	unknown	electrocution
Bbz 19	male	juvenile	Loire River	pullus dead in nest
Bbz 20	female	adult	unknown	illegal shot
Bbz 21	male	subadult	Germany	illegal shot
Bbz 23	female	adult	Germany	drawn in fish farm
Bbz 24	female	subadult	unknown	drawn in fish farm
Bbz 25	male	subadult	unknown	drawn in fish farm
Bbz 28	male	subadult	Germany	dead in health center
Bbz 31	male	adult	unknown	drawn in fish farm

Table 3. Ospreys' characteristics and causes of death analyzed in this study. Osprey numbers corresponds to the chronological sampling order.

### 3.2 Contamination by organochlorine pesticides

Results concerning contamination of otters by OC pesticides are represented in table 4. OC pesticides and especially DDT metabolites were detected in all (100%) of the analyzed otters, confirming the widespread exposure of otter habitat in France to OC pesticides (Colas et al. 2006; Lemarchand et al. 2007, 2010). Mean concentrations of total OC pesticides in otter liver of the whole Loire River catchment reached 2,2 mg.kg<sup>-1</sup> lipid weight, without any statistical variations with the geographical origin of the individuals: the increase in concentrations by going downstream observed in the upper part of the catchment (Lemarchand et al. 2007) was not significant at the whole catchment scale. Differences of various OC pesticides with otter age or sex were not significant. DDT was detected in 17 individuals (33%), confirming quite recent uses of this insecticide, banned in 1973 in France. 15 of these 17 DDT-contaminated otters were coming from the lower part of Loire River catchment. However, DDE was the most abundant of the analyzed DDTs metabolites, confirming the general decrease of otter's exposure to DDT and OC compounds in Europe (Mason, 1998). Lindane constituted the most abundant OC pesticide after DDTs, with quite low concentrations. Aldrin, Dieldrin, Heptachlor and Heptachlor epoxide were very low, often close to the detection limits. Methoxychlor and Endosulfan were never detected in otters. Measured OC pesticides concentrations remained below the available thresholds concerning otter survival (Mason and Macdonald 1993 a,b; 1994). Considering the actual population dynamic in France and elsewhere in Europe, OC compounds are not supposed to constitute an immediate threat to otter conservation.

OC pesticides	Otters (n= 51)					Ospreys (n= 17)					
	Males	Females	Juveniles	Sub adults	Adults & Old	Males	Females	Eggs	Juveniles	Sub adults	Adults
DDT	0,02	0,01	-	0,01	0,02	-	-	-	-	-	-
DDE	1,12	1,85	0,1	1,45	2,01	10,7	0,55	3,40	-	-	1,86
DDD	0,54	0,35	-	0,41	0,60	-	-	-	-	-	-
Lindane	0,11	0,08	0,05	0,15	0,12	-	-	-	-	-	-
Methoxychlor	-	-	-	-	-	-	-	-	-	0,01	0,3
Aldrin	0,05	0,04	-	0,04	0,11	-	-	-	-	-	-
Heptachlor	0,01	0,01	0,01	0,18	0,14	-	-	-	-	-	-
Hepta. epox.	0,01	0,01	0,01	0,01	0,02	-	-	-	-	-	-
Endosulfan	-	-	-	-	-	-	-	-	-	-	-

Table 4. Contamination of otters and ospreys from the Loire River catchment by organochlorine pesticides (mg.kg<sup>-1</sup>).

Results concerning contamination of ospreys by OC pesticides are presented in table 4. OC pesticides were detected in all but 5 of the sampled ospreys, and maximum  $\Sigma$  OC pesticides concentrations in liver reached 10,7 mg.kg<sup>-1</sup> lipid weight. Only DDTs residues (mainly *p,p'*-DDE) and Methoxychlor were found in samples. DDT by itself was never found in ospreys. These two compounds were never found simultaneously in the same samples. Lindane, Aldrin, Heptachlor, Heptachlor epoxide and Endosulfan were never found in samples. Endosulfan is the only OC pesticide never found in otter or osprey samples. Nevertheless, these compounds were noted in previous studies concerning ospreys, particularly concerning Lindane, Aldrin and Heptachlor epoxide (Ewins *et al.* 1999; Henny *et al.* 2003,

2008; Toschik *et al.* 2005). This difference could be related to a different exposure of American ospreys to OC pesticides when compared to European ones, resulting in a higher OC pesticides accumulation pattern in the whole American population. Indeed, American ospreys were exposed to OC pesticides without interruption from the beginning of industrial uses until legal ban. In France, DDT and other OC pesticides were banned before the return of the osprey or at the beginning of population expanding, resulting in a lower and decreasing exposure to contaminants. DDE was detected in 4 individuals (24 %), including 2 eggs from 2 different nests along Loire River and 2 adults, one coming from Loire River. We did not observe any significant variations in OC pesticides concentrations with osprey age, sex or origin. DDE concentrations remained quite low (range 0.0 - 10,7 mg.kg<sup>-1</sup> lipid weight). These values were comparable to those noted by Rattner *et al.* (2004) or Henny *et al.* (2008), and should not be of concern for osprey direct conservation. DDE concentration in available osprey eggs (n=3) reached 0.0, 4,6 and 5,9 mg.kg<sup>-1</sup> lipid weight, respectively. Concerning the latter, the measured values were slightly higher than the 4.2 mg.kg<sup>-1</sup> (measured in wet weight) eggshell thinning threshold cited in the literature (Wiemeyer *et al.* 1988; Henny *et al.* 2008), but these eggs did not show any shell breakage and were not damaged. Methoxychlor was detected in 8 individuals (47%), with low values (range 0.0 - 0.93 mg.kg<sup>-1</sup> lipid weight, see table 1). General Methoxychlor mean reached 0.01 mg.kg<sup>-1</sup> ww, far less than noted by Weber *et al.* (2003) in Germany, where 100% of the sampled ospreys were contaminated by Methoxychlor. Following these authors, we assume this compound is not a direct threat to ospreys.

### 3.3 Contamination by organophosphate, carbamate and pyrethroids pesticides

To a general point of view, contamination of otters and ospreys by the 16 highly toxic cholinesterase inhibitors appeared low and scattered, with only few individuals concerned. Only two otters (4%) and 8 ospreys (47%) were characterized by detectable cholinesterase inhibitors concentrations. Among the OP pesticides analyzed, 7 (Mevinphos, Phorate, Malathion, Parathion, Methidathion, Disulfoton sulfone and Triazophos) were quantified in ospreys and are presented in table 5. Only two OP pesticides (Parathion and Methidathion) were detected in otters, with low concentrations (between 0,02 and 0,03 mg.kg<sup>-1</sup> ww, data not shown). No statistical comparison could have been made concerning otter contamination by OP pesticides. Other OP pesticides analyzed in otter and osprey tissues were never been detected. Carbamates pesticides (Methiocarb and Carbofuran) were not quantified in otters and ospreys during this study, however they were recently noted in intoxicated red kites (*Milvus milvus*) in France (Berny and Gaillet, 2008). It can be assumed that diet of otters and ospreys (based on fish) is less exposed to carbamates pesticides accumulation than other diet types of some terrestrial predators like red kite.

OP pesticides were only measured in subadult and adult ospreys, and never found in eggs or *pulli* during this study. None of the individuals coming from the nesting population showed any OP pesticides contamination. OP pesticides variations with osprey age, sex or origin were not significant. Triazophos, Disulfoton sulfone and Mevinphos were the most frequently detected compounds in ospreys (n=4, 3 and 3, respectively). Phorate and Malathion were detected in only one individual, characterized by the highest diversity of compounds (4 compounds with also Parathion and Methidathion) and by the highest concentrations of total OP pesticides (0,9 mg.kg<sup>-1</sup> ww, see table 5).

Individuals	bbz 19	bbz 3	bbz 7	bbz 8	bbz 9-11	bbz 12	bbz 13	bbz 14	bbz 28	bbz 21	bbz 23	bbz 4	bbz 20	bbz 24	bbz 25	bbz 17	bbz 31
Mevinphos	-	-	-	-	-	-	-	-	-	-	0,03	-	0,05	-	0,3	-	-
Phorate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,02	-
Malathion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0,03	-
Parathion	-	-	-	-	-	-	-	0,4	-	-	-	-	-	-	-	0,8	-
Methidathion	-	-	-	-	-	-	-	-	-	-	-	-	0,02	-	-	0,02	-
Disulfoton sulfone	-	-	-	-	-	-	-	-	-	-	-	-	0,3	-	0,3	-	0,04
Triazophos	-	-	-	-	-	-	-	0,02	0,02	-	-	-	-	0,03	0,03	-	-

Table 5. Contamination of ospreys by OP and CA pesticides (mg.kg<sup>-1</sup> wet weight). Data are organized according to geographic origin of individuals for a better comparison.

As described above, ospreys were generally in good physical conditions (adequate mass and total body fat) and did not show any OP pesticides poisoning sign (e.g. diarrhea, pulmonary oedema, tightened claws, Berny and Gaillet, 2008) during post-mortem examination. Furthermore, some of them were collected during migration flows, and none bird was found with apparent sign of exhaust potentially brought about by contamination consequences. Measured concentrations remained well below toxic doses of cholinesterase inhibitors (documented as about 10 mg.kg<sup>-1</sup> ww) and were not death causal agent of these individuals. Low level of concentrations and of contamination cases frequency should not constitute a threat to the population level, taking into account recent restrictions on OP and CA pesticides uses.

Pyrethroids pesticides residues were never found in any otter or osprey samples (data not shown). The good quality and abundance of samples and the efficiency of the method used avoided methodological bias in pyrethroids pesticides detection.

These results lead to several hypotheses:

- Pyrethroids pesticides may be quickly degraded or metabolized by animals;
- Pyrethroids pesticides may be little concerned by bioaccumulation in aquatic food chains;
- Global accumulation and transfer of recently used pyrethroids is very low for the moment, but is able to raise in the future with increasing uses.

Metabolite of pyrethroids (3-phenoxybenzoic acid 3-PBA) was investigated in osprey eggs of the Washington State, USA (Chu et al. 2007) without being found. Complementary studies are needed to precisely evaluate general contamination of fauna by pyrethroids pesticides. Insect-consumers birds in treated areas (e.g. Eurasian skylark *Alauda arvensis*, common quail *Coturnix c.*) and their bird-eating predators (e.g. Montagu’s harrier *Circus pygargus* or western marsh harrier *C. aeruginosus*) could be used as sentinels for an evaluation of direct transfer of pyrethroids through terrestrial systems first, before generalization of analyses to other systems, like aquatic systems and associate predators.

### 3.4 Contamination by herbicides

As observed for OP and CA pesticides, contamination of otters and ospreys by the 8 analyzed herbicides was generally low and few diversified. Only two otters (4%) and 7 ospreys (41%) showed detectable herbicides concentrations. Of the 10 herbicides analyzed, Metholachlor was the only herbicide detected in otters, on two occasions and with low concentrations (0,02 and 0,05 mg.kg<sup>-1</sup> ww respectively, data not shown). Two herbicides

(Terbuthylazine and Alachlor for 5 individuals each) and fungicide Epoxyconazole (in only one case) were quantified in ospreys (see table 6). None of the ospreys from the nesting population in France showed any herbicide or fungicide contamination. Herbicides variations with osprey age, sex or origin were not significant. Herbicides were not found in osprey eggs during this study. It can be underlined a unique case of contamination by fungicide Epoxyconazole (5,64 mg.kg<sup>-1</sup> ww, see table 6), detected in an osprey from Germany. This individual did not show any particular intoxication sign. As for OP and CA pesticides, concentrations of herbicides measured in tissues and low frequency of herbicide detection leads to a probable weak impact of these compounds on species' conservation. Nevertheless, herbicides were very rarely searched in ospreys and very few data are available in literature for comparison. Chu et al. (2007) reported contamination of osprey eggs by a Dacthal structural isomer, indicating that some herbicides could be accumulated in ospreys with a potential reproductive impact on populations.

Individuals	bbz 19	bbz 3	bbz 7	bbz 8	bbz 9-11	bbz 12	bbz 13	bbz 14	bbz 28	bbz 21	bbz 23	bbz 4	bbz 20	bbz 24	bbz 25	bbz 17	bbz 31
Terbuthylazine	-	-	-	-	-	-	-	0,09	0,83	0,3	-	-	-	0,54	-	0,88	-
Alachlor	-	-	-	-	-	-	-	0,01	-	0,01	-	-	0,01	0,08	-	0,04	-
Epoxyconazole	-	-	-	-	-	-	-	-	-	-	5,64	-	-	-	-	-	-

Table 6. Contamination of ospreys by herbicides and fungicides (mg.kg<sup>-1</sup> wet weight).

#### 4. Conclusion

A large non-invasive program allowed an important sampling of European otter and osprey tissues for various pesticides contamination study. Results showed that otter and osprey could be used as good sentinels of organochlorine and, to a lesser extent, organophosphate pesticides and some herbicides accumulation in aquatic food chains. Carbamates and pyrethroids pesticides were not detected in those top-predators fish-eating species. Organochlorine, organophosphate pesticides and herbicides concentrations remained low and under values of concern for species direct short-term conservation. Regular increase in populations observed since three decades in France seemed to confirm a low impact of global contamination on otter and osprey. Nevertheless, long-term consequences of global contamination on otter and osprey behaviour (*e.g.* prey or habitat foraging, hunting, mating or territory defence), synergies or antagonisms between compounds or potential long-term endocrine disruptors effects of low-concentrated contaminants remains unknown and should be elucidated during future standard monitoring of these sentinels species.

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## **Pesticides in the Modern World - Risks and Benefits**

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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.

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